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14. ABSTRACT

PI Angenent and co-PI Logan have organized the 2012 NA-ISMET and 2014 NA-ISMET meetings at Cornell University and Penn State University, respectively. The objective of the NA-ISMET meetings was to link together researchers and engineers, who work on microbial electrochemical systems or on the interface between microbes and solid electrode materials. In addition, meetings have dissipate new ideas in the fast-emerging field of microbial electrochemical systems. They are meetings where students and post-docs can meet the leading researchers in the

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Report Title

Final Report: North American International Society for Microbial Electrochemical Technologies Meeting

ABSTRACT

PI Angenent and co-PI Logan have organized the 2012 NA-ISMET and 2014 NA-ISMET meetings at Cornell University and Penn State University, respectively. The objective of the NA-ISMET meetings was to link together researchers and engineers, who work on microbial electrochemical systems or on the interface between microbes and solid electrode materials. In addition, meetings have dissipate new ideas in the fast-emerging field of microbial electrochemical systems. They are meetings where students and post-docs can meet the leading researchers in the field and where they can present their work during podium or poster presentations. Left over funding was used to support activities at the international meeting for ISMET that was held in North America in October 2015 at Arizona State University. All these meetings were successful and the next NA-ISMET meeting is held at Stanford in October 2016.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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Welcome

On behalf of the ISMET leadership, the NA-ISMET planning committee, and the Angenent lab, we would like to welcome you to Cornell University for the first North American meeting of the International Society for Microbial Electrochemistry and Technology. We have received a broad range of abstracts describing a variety of exciting research and look forward to interesting talks, posters, discussions, and new ideas! Thank you for joining us in making this meeting a success.

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General Information

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Program

| Monday, October | Monday, October 8, 2012 | | |
|-----------------------------|---|--|--|
| 6:00 - 8:00 PM | Welcome reception | | |
| Tuesday, Octob | Tuesday, October 9, 2012 | | |
| 9:00 - 9:15 AM | Introduction - Dr. Lars Angenent, Cornell University | | |
| 9:15 - 9:45 AM | Keynote Speaker - Dr. Bruce Logan, The Pennsylvania State University | | |
| First Session | | | |
| 9:45-10:15 AM | Invited Presentation - Dr. Caroline Ajo-Franklin, Lawrence Berkeley National Laboratory | | |
| 10:15-10:45 AM | Invited Presentation - Dr. Orianna Bretschger, J. Craig Venter Institute | | |
| 10:45-11:00 AM | Break | | |
| 11:00-11:20 AM | Submitted Presentation - Emily Gardel, Harvard University | | |
| 11:20 -11:40 AM | Submitted Presentation - Zheng Ge, University of Wisconsin-Milwaukee | | |
| 11:40 AM -12:00 | Submitted Presentation - Cynthia J. Castro, The University of Massachusetts-Amherst | | |
| 12:00-1:00 PM | Lunch | | |
| 1:00 - 1:30 PM | Keynote Speaker - Dr. Derek Lovley, University of Massachusetts - Amherst | | |
| Second Sess | ion | | |
| 1:30-1:50 PM | Submitted Presentation - Hiroyuki Kashima, The Pennsylvania State University | | |
| 1:50-2:10 PM | Submitted Presentation - Shunichi Ishii, J. Craig Venter Institute | | |
| 2:10-2:30 PM | Submitted Presentation - Hengjing Yan, The Pennsylvania State University | | |
| 2:30-2:45 PM | Break | | |
| 2:45-3:05 PM | Submitted Presentation- Nathan Samsonoff, University of Toronto | | |
| 3:05-3:25 PM | Submitted Presentation - Li Xiao, University of Wisconsin-Milwaukee | | |
| 3:25-3:45 PM | Submitted Presentation - Andrew D. Jones III, Massachusetts Institute of Technology | | |
| 3:45-4:05 PM | Submitted Presentation - Matthew Yates, The Pennsylvania State University | | |
| 4:05-4:30 PM | Break | | |
| 4:30-6:30 PM | Poster Session | | |
| 6:30-10:00 PM | Dinner | | |
| Wednesday, October 10, 2012 | | | |
| 9:00-9:30 AM | Keynote Speaker - Dr. Korneel Rabaey, Ghent University | | |

| Third Session | | |
|------------------|--|--|
| 9:30-10:00 AM | Invited Presentation - Dr. Daniel Bond, University of Minnesota | |
| 10:00-10:30 AM | Invited Presentation - Dr. Hong Liu, Oregon State University | |
| 10:30-10:45 AM | Break | |
| 10:45-11:05 AM | Submitted Presentation - Jacob J. Weinrich, The University of Massachusetts-Amherst | |
| 11:05-11:25 AM | Submitted Presentation - Huijie Hou, University of California-Santa Barbara | |
| 11:25 - 11:45 AM | Submitted Presentation - Casey Forrestal, University of Colorado-Denver | |
| 11:45 AM - 12:05 | Submitted Presentation - Valerie J. Watson, The Pennsylvania State University | |
| 12:05 - 1:15 PM | Lunch | |
| 1:30-2:00 PM | Keynote Speaker - Dr. Ashley Franks, La Trobe University | |
| Fourth Session | on | |
| 2:00-2:30 PM | Invited Presentation - Dr. Bert Hamelers, Centre of Excellence for Sustainable Water Technology (Wetsus) | |
| 2:30-3:00 PM | Invited Presentation - Dr. Peter R. Girguis, Harvard University | |
| 3:00-3:15 PM | Break | |
| 3:15-3:35 PM | Submitted Presentation - Sudeep C. Popat, Arizona State University | |
| 3:35-3:55 PM | Submitted Presentation - Heming Wang, University of Colorado-Denver | |
| 3:55-4:15 PM | Submitted Presentation - Roland Cusick, The Pennsylvania State University | |

Keynote Speakers

Materials and designs of microbial fuel cells that can be used for wastewater treatment today! (And tomorrow...)

Bruce E. Logan

The Pennsylvania State University, University Park, PA, 16802

Microbial fuel cells (MFCs) are still being described as "showing great promise" or as a "technology of the future". Why is that? Advances in materials, combined with an improved understanding on how to construct MFCs in modular format, indicates that the technology has arrived, and that it is ready for pilot scale applications. In this talk I will review the most promising advances in low cost materials for reactors and summarize the designs that are ready for applications today. Of course there remain many challenges for improving performance and longevity of the materials, and these will need to be addressed as well. MFCs continue to evolve into many new and different types microbial electrochemical technologies. In some cases, these include advances that have increased power densities far above that possible to produce using only domestic wastewater. For example, some of these new systems are based on capturing salinity gradient energy in devices that combine reverse electrodialysis with MFCs, devices that we call microbial reverse electrodialysis cells (MRCs). Others use microbial power and accomplish other objectives, such as water desalination or chemical product production. Based on these material and application advances, MFCs are ready to be scaled up and deployed. So what are we waiting for?

Optimizing Electrode to Microbe and Cell to Cell Electron Transfer for Bioenergy Applications and Electrical Control of Microbial Activity

Derek R. Lovley

University of Massachusetts, Amherst, MA 01003

An understanding of the mechanisms for direct transfer of electrons into anaerobic microorganisms is necessary in order to optimize potential practical applications. Applications include the well-established bioenergy strategy of anaerobic digestion of organic wastes to methane as well as microbial electrosynthesis, a process in which electrons provided directly to cells via a cathode serve as the electron donor for the reduction of carbon dioxide to fuels or other biocommodities. In methanogenic digesters microorganisms can exchange electrons via direct interspecies electron transfer (DIET) and practices that promote DIET accelerate methanogenesis. DIET was initially discovered in the study of a co-culture of two Geobacter species that were adaptively evolved under conditions in which the two species needed to share electrons in order to support their mutual syntrophic growth. Multiple lines of evidence have demonstrated that the Geobacter species do not share electrons via previously known mechanisms such as interspecies transfer of hydrogen or formate. Rather, the cells make direct electrical connections. These electrical connections require the conductive pili of both species. Surprisingly, electron conduction along the pili is via a metallic-like conductivity rather than the electron hopping/tunneling that is characteristic of most biological electron transfer. Genetic manipulation of the pili has demonstrated that the metallic-like conductivity can be attributed to key aromatic amino acids which contribute to the pi-pi stacking observed in the pili structure, consistent with the known mechanism for metallic-like conductivity in synthetic organic conducting polymers. At present, electrosynthesis biofilms are relatively thin, limiting rates of product formation. However, the electron transfer through thick layers of electron-accepting cells that is observed in DIET suggests that it should be possible to enhance the growth of cathode biofilms under the appropriate conditions. We have recently developed tools for the genetic manipulation of Clostridium Ijungdahlii, a microorganism effective in electrosynthesis, and have used these tools to develop strains capable of producing novel products from carbon dioxide. A better understanding of how electrons enter cells during electrosynthesis and DIET is expected to lead to the optimization of bioenergy processes and could enable the development of new strategies for controlling microbial activities with electrical signals.

Combined fermentation and extraction in a bioelectrochemical system

Korneel Rabaey, Stephen Andersen, Nina Voulis and Tom Hennebel

Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Ghent, Belgium

Electrical current can be used to alter the outcome of fermentations. This can be achieved by discharging electrons at an anode, as demonstrated in glycerol to ethanol fermentations. Alternatively, electrons can be directed towards fermenting microorganisms at a cathode typically leading to more reduced product outcomes. Next to impacting the fermentation itself, bioelectrochemical systems can be used to harvest the typically anionic end products of mixed culture fermentations. Indeed, by placing an anion exchange membrane between the cathode and the anode, the electrical current will drive a flux of e.g. carboxylates towards the anode. Finally, the electrical current also causes hydroxyl formation in the cathode, thereby decreasing the need for pH control in case the fermentation leads to fatty acid toxicity. In my presentation I will provide an overview of results achieved to date for cathode driven fermentations, particularly in the context of glycerol fermentation, chain elongation and in situ extraction.

Geobacter sulfurreducens as a Model for Electrode Utilising Biofilms

Ashlev E. Franks^{1,2}

The formation of specialized biofilms on an electrode surface is fundamental to the functioning of microbial electric systems (MES). In MES, the electrode-associated biofilms from a wide variety of environmental inoculants are often dominated by Geobacter species. The genetically manipulatable strain Geobacter sulfurreducens produces a current density equal or greater than mixed or pure cultures and has become a model for the study of electrode associated biofilm processes. Current producing electrode-associated biofilms of G. sulfurreducens are greater than 50 µm thick and spatially heterologous. The development of real-time imaging microbial fuel cell allows the nondestructive spatial and temporal imaging of G. sulfurreducens biofilms in situ. Metabolic staining indicates that the entire biofilm is metabolic active, demonstrating the ability to transfer electrons at distances greater than 50 um. A pH sensitive fluorometric dye demonstrated that while all members of the biofilm are metabolically active, a potentially inhibitory proton gradient forms within the biofilm specifically due to extracellular electron transfer. Spatial transcriptional analysis within the biofilm revealed a large number of genes down regulated in the outer layer with roles in cell metabolism and growth, however differences in transcript abundance is quite low. The creation of a short-half life green fluorescent protein reporter plasmids now allows the spatial and temporal analysis of gene expression within the current producing biofilms in real time. Differences between gene expression and protein localization were observed comparing gene expression to protein localisation using gold-labeled antibody probing of imbedded current producing biofilms. Differences in distribution were observed for proteins thought to be essential for power production indicating possible spatial post-transcriptional regulation. Theses results highlight the unique and highly heterologous nature of current producing biofilms and are expected to lead to better strategies for optimizing the function of MES and broadening their applications.

¹ Department of Microbiology, University of Massachusetts, Massachusetts, USA.

² Department of Microbiology, La Trobe University, Melbourne, Victoria, Australia

Invited Presentations

Making New Electroactive Microbes Using Synthetic Biology

Caroline M. Ajo-Franklin^{1*}, Heather M. Jensen¹, Cheryl P. Goldbeck¹, Michaela A. TerAvest², Matthew Helper¹, Largus T. Angenent²

¹Lawrence Berkeley National Laboratory, Berkeley, CA 94720 and ²Cornell University, Ithaca, NY 14853

By transplanting extracellular respiration into heterologous organisms, we seek to create engineered organisms whose capabilities in chemical energy conversion and chemical synthesis exceed those found in nature. In this talk, I will describe genetic strategies to improve the synthesis and function of one such electron transfer pathway, the Mtr pathway from *Shewanella oneidensis*, into *Escherichia coli*. In addition to being able to reduce soluble and solid Fe, some Mtr-containing E. coli strains can generate more current per cell in an electrochemical cell their parent strain. Interesting, electrochemical function of these strains is not well-correlated with the number of electron transfer pathways per cell. Thus, our work both indicates it is feasible to re-program organisms to respire with electrodes and highlights the importance of system-level optimization of genetic circuits to successfully accomplish this.

Meta-omics and Microbial Fuel Cells

Orianna Bretschger, Shino Suzuki Ishii, and Shun'ichi Ishii

The J. Craig Venter Institute, San Diego, CA 92121

Meta-omics based approaches including metagenomics, metatranscriptomics and metaproteomics have enabled researchers to more deeply explore the genetic potential and functional relationships within complex microbial communities. Several research efforts have successfully employed these techniques to characterize microcosms including marine, soil, and human ecosystems. However, only a few studies have utilized 'omics' tools to evaluate microbial dynamics in microbial fuel cell (MFC) systems. Here we present a summary of different approaches and analyses tools that have been recently employed to understand the microbial taxonomic and functional complexities that exist in MFC systems. Further, we will address how these data sets can be used to better understand and optimize MFC systems for practical applications and extract useful fundamental knowledge about microbial ecosystems.

Development and Evaluation of Electrode and Separator Materials for Microbial Fuel Cells

Hong Liu

Oregon State University, Corvallis, OR 97331

The performance and cost of electrode and separator materials are critical for practical applications of microbial fuel cell (MFC) technology, especially in wastewater treatment. This talk will cover recent developments of MFC materials with focus on the performance and cost reduction of separators and non-Pt cathodes. Some essential techniques and common issues for evaluating electrode materials will also be discussed.

BES: From Energy to Resource Recovery

Bert Hamelers

Centre of Excellence for Sustainable Water Technology, 8934 CJ Leeuwarden

Due to the growing world population and increasing level of wealth, there is a strong growing demand for both energy and materials. BESs are intensively researched to extract energy from the wastewater either as electricity or hydrogen. Recovery of material from wastewater using BES is another interesting application. Materials may have a higher value making application of a BES system attractive. Two applications will be discussed copper and N removal from urine.

Biogeochemical relevance of extracellular electron transfer in nature, and inspiration for energy production and bioelectrosynthesis

Peter R. Girguis

Harvard University, Cambridge, MA, 02138

Extracellular electron transfer enables microbes to interact with solid-phase (or spatially remote) oxidants and reductants. EET has been well-studied in cultivated microbes, e.g. heterotrophic iron-reducing δ -proteobacteria. The relevance of EET in nature, however, and its impact on biogeochemical cycles remains poorly constrained. Anaerobic environments typically host microbial communities that are involved in numerous biogeochemical cycles, and those capable of EET may have a bioenergetic advantage. Here we present data on the population structure, functional potential and activity of EET-associated microbes in a variety of naturally-occuring anaerobic environs. These data suggest that microbial EET is widespread among microbes, and likely has a pronounced influence on key biogeochemical cycles. The findings presented herein provide a plausible explanation for some previously inexplicable biogeochemical phenomena, underscore the need for further investigation into the relevance of EET to global biogeochemical cycles, and reveal opportunities for industrial applications.

Oral Presentations

Duty Cycling Influences Current Generation in Multi-Anode Environmental Microbial Fuel Cells

Emily J. Gardel^{1*}, Mark E. Nielsen¹, Phillip T. Grisdela Jr.², Peter R. Girguis¹

¹Harvard University, Cambridge, MA 02138 ²Dartmouth College, Hanover, NH 03755

Improving microbial fuel cell (MFC) performance continues to be the subject of research, yet the role of operating conditions, specifically duty cycling, on MFC performance has been modestly addressed. We present a series of studies in which we use a 15-anode environmental MFC to explore how duty cycling (variations in the time an anode is connected) influences cumulative charge, current, and microbial composition. The data reveal particular switching intervals that result in the greatest time-normalized current. When disconnection times are sufficiently short, there is a striking decrease in current due to an increase in the overall electrode reaction resistance. This was observed over a number of whole cell potentials. Based on these results, we posit that replenishment of depleted electron donors within the biofilm and surrounding diffusion layer is necessary for maximum charge transfer, and that proton flux may be not limiting in the highly buffered aqueous phases that are common among environmental MFCs. Surprisingly, microbial diversity analyses found no discernible difference in gross community composition among duty cycling treatments, suggesting that duty cycling itself has little or no effect on gross community composition. Such duty cycling experiments are valuable in determining which factors govern performance of bioelectrochemical systems and might also be used to optimize field-deployed systems.

Reducing Effluent Discharge and Recovering Bioenergy in an Osmotic Microbial Fuel Cell Treating Domestic Wastewater

Zheng Ge *, Qingyun Ping, Li Xiao, Zhen He

University of Wisconsin-Milwaukee, Milwaukee, WI 53211

Osmotic microbial fuel cells (OsMFCs) are an emerging concept that integrates forward osmosis into an MFC for simultaneous wastewater treatment, bioenergy recovery and water extraction. Previous studies of OsMFCs have demonstrated the proof of concept and examined different draw solutes as catholytes. This study investigated the performance of an OsMFC treating actual domestic wastewater. It was found that the OsMFC achieved water flux of 1.06-1.49 LMH and reduced wastewater effluent by 24.3-72.2% depending on hydraulic retention time. Increasing the recirculation rate of the catholyte from 100 to 3000 mL/min slightly improved water flux to 2.15 LMH. The low water flux was likely due to inefficient reactor configuration, membrane fouling and concentration polarization. The OsMFC constantly produced bioelectricity and achieved a maximum power density of 28.2 W/m3 from acetate solution or 4.5 W/m3 from domestic wastewater. Preliminary energy analysis suggested that the OsMFC had a potential to produce more energy than that was consumed by the pumping system, thereby creating an energy-neutral wastewater treatment system. The analysis of membrane fouling after more than 100-day operation of the OsMFC using electrochemical techniques, imaging and water flux test revealed microbial fouling and abiotic scaling on the surface and inside the FO membrane.

Decentralized Organic and Nitrogen Removal from Domestic Waste in Rural Ghana with a **Microbial Fuel Cell**

Cynthia J. Castro*, Joseph E. Goodwill, Caitlyn, S. Butler

The University of Massachusetts Amherst, Amherst, MA 01003

Sanitation solutions are needed in developing countries to reduce water borne illnesses. Microbial fuel cells (MFCs) with low biomass growth and electricity yield are a promising energy efficient technology for waste treatment. Although many bench-scale MFC systems have sustained power production, only few studies have examined these systems at a pilot scale. The goal of this study was to develop a costefficient pilot scale MFC that would remove organics and nitrogen compounds directly from human waste and to deploy the pilot design in rural Ghana. An MFC was incorporated into the design of a simple composting latrine that is commonly found in rural regions of Africa allowing for utilization of local materials, labor and operation. In the proposed system, solid wastes are filtered into a composting chamber and the remaining liquid stream is passed to an MFC. The MFC design consisted of three compartments: the anode chamber (28L Liquid), where COD was oxidized by anode biofilm, an intermediate nitrification chamber (45L Liquid) supporting aerobic microorganisms, and the cathode chamber (28L Liquid), where nitrate was reduced to inert nitrogen gas by a cathode biofilm. The chambers were hydraulically partitioned, eliminating the need for a proton exchange membrane, and inexpensive granular graphite was used as the electrode material. This 'single-seat' MFC could accommodate a population of 100 people. One of the primary advantages of the MFC latrine system design is that it can be retrofitted to existing latrines with minimal disruption to existing sanitation programs. Prior to the system construction in Ghana in May 2012, an analogous, lab-based pilot MFC was constructed. The MFC anode biofilm oxidized 74 mg COD/L-d, achieving over 98% percent removal. The total nitrogen removal rate was 34 mg N/L-d or 68% of nitrogen removal. Power production was low, 2.5 mW/m³. A low power density was expected. Significant factors identified as limiting power production in this system were ohmic losses due to the electrode material and conductivity of the waste stream. Though power production was low, the system was able to power a LED light, a useful resource in a rural, developing area where electricity is not reliable.

Facultative Nitrate Reduction by an Exoelectrogenic Denitrifier, Geobacter metallireducens, as a Competitive Reaction to Anode Reduction in Microbial Electrolysis Cells

Hiroyuki Kashima* and John M. Regan

The Pennsylvania State University, University Park, PA 16802

Bioelectrochemical systems are beginning to be designed for integrated nitrogen removal (e.g. nitrification and denitrification) to combine sustainable wastewater treatment and renewable energy production. Since some exoelectrogens can facultatively reduce nitrate, nitrate could act as a competitive electron acceptor to anode for exoelectrogens, and it is important to understand the critical conditions controlling this facultative metabolic shift to develop reliable nitrogen removal strategies in bioelectrochemical systems. Unlike the well-studied case of dissolved oxygen (D.O.) effects on the facultative metabolic shift between aerobic respiration and nitrate respiration in anoxic denitrification processes, the conditions that determine anode versus nitrate reduction are unknown.

This research is focused on understanding the competition between nitrate reduction and anode reduction for an exoelectrogenic denitrifying bacterium (Geobacter metallireducens) in microbial electrolysis cells (MECs), to determine the critical conditions controlling these facultative processes. We hypothesize that G. metallireducens biofilms preacclimated on graphite anodes choose their electron acceptor (anode or nitrate) as a function of the anode potential and/or nitrate concentration.

In batch tests with acetate-fed two-chamber MECs, G. metallireducens biofilms preacclimated on polished graphite anodes maintained at a potential of 0 mV vs. standard hydrogen electrode as the sole electron acceptor immediately performed both nitrate and anode reduction in nitrate-spiked batch cycles. This competitive reaction resulted in lower anode reduction rates and smaller electron distributions to anode reduction. In addition, the presence of nitrate in the anode chamber resulted in increased suspended biomass relative to controls without nitrate, and this nitrate-induced suspended growth decreased coulombic recovery as well as nitrate reduction by anode biofilm. Furthermore, in batch tests with acetate-fed single-chamber mini-MECs and 0.7 V applied voltage, the circuit-recovered charge decreased with increased nitrate concentration. Further tests will be conducted at various anode potentials to understand the critical conditions controlling these facultative metabolic processes.

Microbial and genetic response for facilitating electricity generation in wastewater-fed microbial fuel cell

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Microbial fuel cells (MFCs) are devices that recover electricity from organic matter via microbial biocatalytic reactions. Optimizing conditions for higher current production will lead to accelerated organic matter-degradation. In this study, electrochemical, microbial, and genetic features were analyzed in wastewater-fed MFCs operating under different current producing conditions. We used primary clarifier effluent from a sewage treatment plant as carbon and inoculum source for MFCs.

Three different conditions were explored throughout one year of operation: 1) MFC operation using an external resistor; 2) poised-potential operation of the anode for accelerating current generation; and 3) open circuit operation with no current generation. The wastewater treatment was notably faster under poised potential (higher current) conditions. We also analyzed microbial community dynamics of electrogenic anode biofilms using 16S rRNA gene sequencing on clone library method. The frequency of phylotypes classified as family Desulfobulbaceae and Geobacteraceae increased under higher current generating conditions, indicating those were important electricity generators.

Further, a metagenomic analysis was also conducted using next generation sequencing (Illumina GIIx and 454 Ti) to characterize the gene resources within the enriched anodic microbial community after long-term MFC operation. The assembly and taxonomic assignments suggested that one Desulfobulbaceae strain, one Desulfobacteraceae strain, and one Methanosarcinaceae strain were the most dominant species within the community. The functional annotation of those three strains revealed that Desulfobulbaceae strain has various types of multi-heme c-type cytochromes, Desulfobacteraceae strain has dissimilatory sulfate reducing pathway(s), and Methanosarcinaceae strain has methanogenesis pathway(s), respectively. These analyses indicate that the Desulfobulbaceae strain have been a potential electricity generator using c-type cytochromes as a mechanisms for electron transfer. This finding also correlates directly with our microbial community analysis, which showed that Desulfobulbaceae was abundant and active taxon for current generation within the community. These works showed us in detail overview of what was happened within the electrogenic microbial community. This work was supported by NSF-BBBE (award No. 0933145) and NSF ecosystem cluster (Award No. 0918983).

Cathode-Influenced Metabolic Flux Distribution in Exoelectrotrophic Clostrida

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Fermentation processes for the production of industrially valuable organic compounds have been outcompeted by petrochemical synthesis routes around the middle of the last century. However, due to the limited supply of petroleum and an environmentally driven interest in the use of renewable resources as feedstocks for the production of chemicals, biosynthesis routes are becoming increasingly attractive. In bioelectrochemical systems (BESs), it was recently demonstrated that acetogens could synthesize acetate from carbon dioxide on the cathode electrode by consuming electrical current, which was called microbial electrosynthesis (MES). Since various renewable sources could be used for current production, including wastewater, solar energy, and wind energy, MES provides a new technological strategy for biochemical production.

Some Clostridium species produce more diverse fermentation products than acetogens, such as ethanol, butyrate, and butanol. In addition, some clostridia were already reported to be exoelectrogenic, but they do not have the Wood-Ljungdahl pathway for carbon dioxide fixation. In this work, we investigated the exoelectrotrophic activity of some clostridia and whether cathode oxidation influenced the flux of carbon through their fermentative pathways for the production of various products. We found the Clostridium species we selected could accept electrons from the cathode at a poised cathode potential around -400 mV (vs SHE) using glucose as the carbon source and primary electron donor. Diverse and energy-enriched products were also detected during this process. Current work is focusing on the effect of cathode-derived supplemental electrons on the biosynthesis of organic products with clostridia.

Cogeneration of Biofuel and Electricity through an Evanescently-Excited Photosynthetic **Microbial Fuel Cell**

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Cyanobacteria are photosynthetic microorganisms that play a significant role in the biosphere as primary energy producers. Due to their minimal energy requirements of little more than sunlight, water and carbon dioxide [1], they are receiving significant attention as a source of sustainable bioenergy. Both wild and genetically modified forms have been investigated as a source of biofuels [2] and as biocatalysts in microbial fuel cells to generate electricity [3]. Growth under evanescent excitation and direct synthesis of biofuel from cyanobacteria have been demonstrated, promising substantial increases in biofuel production density [4, 5, 6].

Here we propose a combined approach using a photosynthetic microbial fuel cell (PMFC): a densely populated photobioreactor that cogenerates electricity and biofuel. High cyanobacteria density is achieved by evanescent photo-excitation, which provides targeted light delivery to waveguideimmobilized cells, allowing layers to be grown in close proximity [4, 7].

In the native state, photo-excited chlorophyll a in photosystem II hydrolyses water to release electrons into the electron transport chain, which terminates in ATP (adenosine tri-phosphate) production for cellular energy storage. The electron can instead be harvested partway through the chain in photosystem I into a conductive anode supporting the biofilm for external electrical power generation.

Within the PMFC, hydrogen ions and oxygen gas produced from the hydrolysis of water recombine with the captured electron at the cathode to produce water. A PMFC has been constructed with a 100 nm gold anode on glass and a 360 µm thick carbon paper cathode. The cyanobacterium Synechococcus elongatus was used as the biocatalyst in a PDMS cavity on the anode. Direct incident lighting was provided by a tungsten-halogen source. Positive light response has been demonstrated.

Initial results indicate a power density on the order of 1.6 nW/m² at a 10 M Ω load and a maximum current density of 2.6 $\mu A/m^2$ at 680 $k\Omega$. Current work focuses on the implementation of evanescent excitation in conjunction with current collection, and biofuel production.

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Production of Electricity and Algal Biomass in an Integrated Photo-Bioelectrochemical System **Treating Artificial Wastewater**

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Wastewater treatment plants play a critical role in environmental protection but the operation of those plants consumes intensive energy. Microbial fuel cell (MFC) technology is a promising and alternative approach to secondary treatment with functions of energy recovery from organic wastes. Algal treatment of wastewater has a long history of application to remediate wastewater, especially removal of nutrients and heavy metals. The objective of this work is to achieve the removal of both organic wastes and nutrients from wastewater with reduced energy consumption, while producing bioelectricity and biomass (algae) through integrated bio-electrochemical and microbiological processes. We have developed a treatment system involving a unique integration of MFCs into an algal bioreactor, leading to a potentially more efficient system for both waste treatment and bioenergy production. In this integrated photo-electrochemical (IPB) system, wastewater is fed into the MFCs where organic contaminants are converted into bio-electricity; the remaining nutrients are then discharged into algal bioreactor for algal growth which strips nutrients out of the water before the treated effluent is released for final tertiary treatment. Installing MFCs inside an algal bioreactor can have algae producing oxygen via photosynthesis and this oxygen is used by MFCs for their cathode reactions, thereby reducing the need for aeration. Our bench IPB system has been operated for more than one year and produced a maximum power density (2.2±0.2 W/m³) under illumination. The IPB system removed 93% COD, 82% phosphorus and 98% ammonia. The removal of total nitrogen is more than 50%, due to the remaining nitrate. We analyzed microbial community on the cathode electrode and in the algal growth solution, and identified the dominant bacterial and algal species. Further analysis of syntrophic or competitive relationship between bacteria and algae is in the process and the results will be presented in the ISMET meeting.

A Microfluidic Platform for Evaluating Anode Substrates for Microbial Fuel Cells

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Microfluidics is a useful platform for studying microbial processes. The high surface area to volume ratios can increase throughput and decrease transport times for chemical and physical stimulation while transparent substrates provide easy optical access. In using microfluidics, scale and material selection must be representative of the full-scale system. In this work, we show how material selection impacts growth of electroactive biofilms for microbial fuel cells. While commercial microbial fuel cells typically use graphite as the film substrate, it is difficult to rapidly prototype micro-patterned graphite. In this work, pyrolytic graphite sheets (PGS), sputtered carbon on titanium, gold on titanium, titanium, and indium tin oxide are integrated into a microfluidic microbial fuel cell. The microfluidic microbial fuel cell uses Geobacter sulfurreducens fed with acetate in a phosphate buffer media. Ferricyanide is used as the catholyte so that the system is anodically limited. Current versus time and open circuit voltage versus time show differences in biofilm growth and electrochemical activity. Current density on 0.35 x 20 mm PGS was 20 mA/m² at +0.2 V/Ag/AgCl after 6 hrs. Polarization curves and power density curves highlight the final effects of growth on differing substrates. Finally, differences in biofilm morphology will be probed with fluorescence microscopy and scanning electron microscopy. Results of this study will advance our understanding of the growth dynamics of electrochemically active biofilms.

Pre-enrichment with *Geobacter sulfurreducens* to Enhance Performance of Microbial Electrolysis Cells

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Biofilms are the motor of bio-electrochemical systems (BESs). For this reason, the microbial composition of the biofilm is very important, particularly near the anode surface. The anodic community influences the properties of biofilms such as conductivity and the ability to break down various substrates. It has been shown that *Geobacter sulfurreducens* can create a conductive matrix on an electrode surface, which can enhance the bio-electrochemical reduction of the anode to produce biofuels in BESs. We hypothesized that pre-enrichment of the anode with a biofilm of *G. sufurreducens* would improve current generation using glucose and domestic wastewater, based on the microbes on top of this electrogenic biofilm releasing fermentation end products that could be used for current generation. In a glucose-fed BES, pre-enrichment of the anode with a *G. sulfurreducens* biofilm should therefore have a positive impact on performance compared to the control (anode was not pre-enriched). However, there was no effect of pre-enrichment when wastewater was the substrate unless the biofilm was first inactivated. Not only is the community of the anode important, but the structure of the biofilm can also give insights into specific microbial interactions within the anodic biofilm. To study this, a method was developed to visualize the cross section of an intact biofilm using a combination of LR White hydrophilic resin, microtoming, and fluorescent in-situ hybridization (FISH).

Kinetics of nitrate-reduction and nitrite-accumulation in a denitrifying biocathode

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Microbial fuel cells (MFCs) can simultaneously oxidized dissolved organic compound in the anode and reduce oxidized nitrogen (denitrification) in the cathode while producing electricity from municipal wastewater. These MFCs have been well studied over the past few years in bench-scale conditions. Previous studies have explored denitrification intermediates; however, nitrite accumulation has not been reported in MFC biocathodes. Additionally, rate parameters for denitrifying biocathodes have not been determined. In this study, denitrifying biocathodes were observed to accumulate significant nitrite. Batch kinetic parameters for nitrate and nitrite reduction were determined for two identical twochamber membrane-separated microbial fuel cells with denitrifying biocathodes that accumulated significant nitrite. Cathodes were operated in batch mode and the liquid volume was recirculated at 30 mL/min to promote mixing. Initial nitrate concentrations ranged from 11.5-53 mg NO₃-N/m² cathode. A constant acetate concentration 1.184 g COD/m²-anode was continuously fed to the anode at .25 mL/min to prevent substrate-limiting conditions in the anode. The biofilm was assumed to be a steady state monolayer of bacteria, with a biomass density of .001 mg biomass/cm² cathode surface area. Both reactors showed nitrite reduction after complete nitrate reduction. The average peak nitrite accumulation was 70.27% (standard deviation 1.25%) of the initial total nitrogen concentration. The kinetic data was determined using a hyperbolic regression analysis software package, Hyper.exe version 1.1. For nitrate reduction, the half-saturation rate constant (K_{NA}) was 1.70 mg NO_3 -N /m², with a maximum utilization rate (q_{max, NA}) of .078 mg NO₃-N/mg biomass-day. For nitrite reduction, the half saturation rate constant (K_{NI}) was 5.98 mg NO_2 -N/m², with a maximum utilization rate ($q_{max, NI}$) of .075 mg NO₂-N/mg biomass-day. Though these parameters are unique for this nitrite-accumulating denitrifying biocathode community, determining kinetic parameters is important in designing full-scale reactors and predicting biocathode performance.

Oligoelectrolytes Facilitate Electron Transfer in Microbial Fuel Cells

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Microbial fuel cells (MFCs) rely on electron transfer from inside of cell membrane to the chargecollecting anode through respiration chains of microbial metabolism. ^{1, 2} The efficiency of the electron transfer step constitutes an important barrier for improving performance. Recently, conjugated oligoelectrolytes (COEs), with combination characteristics of organic semiconductor and polyelectrolyte, have shown to support electron transfer in MFCs. Different from soluble mediators assisting electron transfer through diffusion in/out of cell membrane, all the evidence of improving electron transfer is consistent with strong binding across the cell membrane. ³ Studies of MFCs with COEs provides insight into the nature of microbial metabolism as well as microbe-electrode interface, an area of research for which many basic science questions remain poorly answered.⁴⁻⁷

We studies E. coli MFCs with two series of COEs to help understand that how molecular variations (number of repeats, length of molecules, side group activities) would lead to different levels of molecule-cell membrane interaction, thus contribute to varied electron transfer responses in MFCs. In this contribution, we take the advantage of a recently developed microbial fuel cell array,8 where 24 miniature MFCs are integrated on the same device, to perform highly reproducible parallel studies in a high throughput fashion. The nature of the homogeneous E-beam evaporated Au electrode surfaces provides uniform cell-electrode interaction interface for all the MFCs. This high-throughput screening prototype is highly suitable to be used as an examination platform, that to what degree molecular variations influence the electron transfer process at the microbe-electrode interface. Screenings of these compounds with E. coli MFCs showed positive power responses than the control unit without COE added, with the highest one showing 24.7-fold power improvement. The fluorescent properties of the COE molecules allow biofilm formation on anode electrode being directly confirmed with confocal microscopy without any tedious staining process. The synthesized COEs provide a new vision as membrane-bonded mediators to assist transmembrane electron transfer for microorganisms as well as medium for microbe-electrode interaction studies.

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Understanding and Solving the Key Challenges in Microbial Desalination Systems

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Microbial desalination cell (MDC) was introduced in 2009 as a new microbial electrochemical system for simultaneous wastewater treatment, desalination, and energy production. Within several years of development, many lab-scale studies have demonstrated interesting and promising results. However, some main challenges have been raised during the years that remain to be resolved. This presentation focuses on our efforts in understanding and addressing the key problems associated with traditional 3chamber MDC processes. We will focus on the most recent findings that have not been published. 1) Traditional MDCs remove salts from the middle chamber by moving them to the anode/chamber chamber, which actually results in an increase of the volume of saline water. By integrating the capacitive deionization concept, we developed two new systems that addressed this salt migration problem and increased desalination efficiency by more than 25 times. By removing anion exchange membranes, we allowed the free transfer of protons and alleviated pH fluctuation problems. 2) We used advanced tools and characterized the ion transfer behaviors and membrane fouling and scaling mechanisms during long-term MDC operation and found that the cations are the main reasons causing membrane and reactor performance drop. We also characterized the microbial community in MDC systems. New findings will be presented to alleviate such concerns.

Performance and Characterization of Activated Carbon Powders for Air Cathode MFCs

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Activated carbon is a promising alternative catalyst to platinum in air cathode microbial fuel cells (MFCs) due to the low cost of this material. Commercially prepared activated carbons made from peat, hardwood, coconut shell, bituminous coal, and phenolic resin were studied. Pore volume and surface area were analyzed for each sample and potentiometric titration was used to determine the amount and type of surface oxygen functional groups present on each of the carbons. The performance of the catalysts for the reduction of oxygen in a neutral pH solution was studied using rotating disk electrodes. The activated carbons made from a peat precursor, which had a mix of micro- and meso- pores and a comparatively modest surface area (800 m²g⁻¹) and pore volume (0.55 mlg⁻¹) and the least amount of oxygen functional groups (0.21 mmolg⁻¹), achieved the largest limiting current density and lowest overpotential, whereas a mostly mesoporous hardwood derived activated carbon, which had a higher surface area (1000 m²g⁻¹) and pore volume (1.1 mlg⁻¹) and the most oxygen functional groups (0.94 mmolg⁻¹), had the lowest limiting current and highest overpotential of the carbons tested.

On Cathodic Potential Losses in Microbial Fuel Cells

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Microbial fuel cells (MFCs) provide a tremendous opportunity for achieving sustainable wastewater treatment, since the energy present in the organic pollutants can be directly converted to electrical power. The theoretical available voltage in MFCs is ~1.1 V when using acetate as the electron donor and oxygen as the electron acceptor, but laboratory experiments normally yield only 0.2-0.4 V at appreciable current densities. The anode contains a biological catalyst (i.e., anode-respiring bacteria) that is often perceived as being rate-limiting, but the anode reaction results in only a ~0.25 V potential loss under maximum current densities (~5-10 A/m²). Instead, the largest fraction of the potential losses (often >60% or >0.6 V) at equal current densities occurs at the cathode, where a metal catalyst, usually platinum, is used to reduce O2. This may seem surprising, since metal catalysts in chemical fuel cells allow obtaining current densities that are >1000 higher. In this talk, we present the latest results from our laboratory from experiments designed to thoroughly understand the origin and nature of cathodic potential losses in MFCs. We show how Nernstian concentration losses due to accumulation of OH⁻ locally on the cathode lead to most of the typically observed cathodic potential losses in MFCs. OH is a product of the O₂ reduction reaction mechanism, which has not often been considered dominant before in MFCs. OH accumulates at the cathode because of its poor transport through the cathode catalyst layer and diffusion boundary layer at the cathode-electrolyte interface. Our results show that the local cathode pH, even when a membrane is not used to separate the anode and the cathode, can easily reach >12 at current densities of 5-10 A/m², representing significant potential loss (~0.3 V). Considering this, we suggest various options for improving cathode performance in MFCs. These include replacing Nafion as the catalyst binder with an anion conductive binder that can better transport OH out of the catalyst either directly or via enhanced transport on OH carriers such as phosphates and carbonates, which too are anionic.

Maximizing Microbial Fuel Cell Energy Output by Active Harvesting

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The research focus of microbial electrochemical systems has been shifting from electricity production to other functions, such as biochemical production, desalination, and remediation. The low direct power output from microbial fuel cells has been one primary reason. Here we present a new strategy of energy harvesting from microbial fuel cells, which our preliminary results have showed can dramatically increase energy output. We call this hysteresis controller based energy extraction approach active harvesting, because it does not use charge pumps or resistors, and it can extract energy at any MFC operating point, especially at the peak power density point for maximum energy harvesting. It can also be operated at high current point for more efficient electron delivery for biochemical synthesis and desalination. Our recent findings demonstrate that within 18-hour test, the energy gained from the active harvesting circuit was 76.8J, 74 times higher than that from a commonly used charge pump (1.02J). The energy recovery efficiency was also 21 times higher in the harvesting circuit. Not yet published, we will present our recently developed digital tracking and harvesting system that can track and harvest the maximum MFC energy in real-time. We will also present our newest findings on how to harvest energy more efficiently based on different various inductances, duty ratios, and frequencies.

Electrochemical Investigation of Symbiotic Power Generation in Microbial Reverse Electrodialysis Cells

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Microbial reverse electrodialysis cells (MRCs) are hybrid technologies that combine microbial fuel cell technologies with integrated reverse electrodialysis (RED) stacks. These systems can be used to simultaneously harvest energy from nearly inexhaustible sources of wastewater and naturally occurring or engineered salinity gradients. MRCs make use of the biological current generated from organic matter by bacteria and salinity gradient voltage and ionic current of the RED membrane pairs. It was shown that a RED stack containing five pairs of high and low concentration salt solutions can increase power generation from MFC electrodes by an order of magnitude. Observationally, MFC electrode power increased because anode and cathode potentials were maintained near open circuit values with increased current. The source of this electrode potential flattening remains unknown. To investigate this phenomenon, we have varied the number of RED cell pairs within the MRC from 1 to 10 in order to monitor performance with different numbers of membrane pairs. In this presentation, I will provide the resulting data on the analysis of these variable numbers of stacks using electrochemical impedance spectroscopy (EIS), linear sweep voltammetry (LSV), and polarization curves. These results will shown how membrane stacked systems can be tailored to specific wastewaters in order to optimize performance.

Poster Presentations

Improvement of Microbial Fuel Cell Performance using a Saline Catholyte Solution

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Microbial fuel cells (MFCs) are devices that generate electricity from wastewater while simultaneously treating wastewater. The main challenges for constructing practical MFCs include an increase in power and recovery of electrons from the substrate (Coulombic efficiency). Oxygen cross-over from cathode to anode can significantly decrease the performance and power densities of air-cathode MFCs. Also, it has hindered the use of lower-cost carbon mesh anode. In this study, a two-chamber MFC containing anion exchange membrane was constructed with high concentration of NaCl as a catholyte solution. The membrane was used to inhibit oxygen crossover. Normally, power is greatly reduced with the membrane. However, the high salinity solution in cathode chamber resulted in low ohmic resistance while achieving high mass transfer resistance of O2 to anode by water chamber. Also, chloride ions helped to maintain charge balance between anode/cathode chambers. Using a 120 mM NaCl solution in the cathode chamber, the maximum power density was only slightly decreased (~17%) compared to that obtained with 50 mM PBS, even though the system contained a membrane between the two chambers. The maximum power output was 491 mW/m² with 250 mM NaCl which was only slightly less than that of the single chamber MFC (577 mW/m²). However, the coulombic efficiency was greatly improved (77~109%) with NaCl catholyte due to the high O₂ transfer resistance. Electrochemical impedance spectroscopy (EIS) data showed that by adding the 240 mM of NaCl, the sum of membrane resistance (R_m) and solution resistance (R_s) decreased from 52 Ω to 23 Ω which was also lower than R_s of single chamber MFCs. Our results show that high concentration of NaCl can significantly increase the performance of air-cathode MFCs by reducing oxygen cross-over from cathode to anode chamber as well as lowering internal resistance in the cathode chamber.

Electrochemical Characterization of Novel Anode-Respiring Bacteria Producing High Current **Densities Under Saline and Alkaline Conditions**

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Microbial electrochemical cells (MXCs) rely on anode-respiring bacteria (ARB) to catalyze the oxidation of organic compounds coupled to the liberation of electrons to a solid electrode. MXCs have been indispensable in elucidating key facets of anode respiration in model ARB such as Geobacter sulfurreducens and Shewanella oneidensis. Nevertheless, there is an untapped potential for MXCs to expand the repertoire of efficient ARB by exploring those with different optimal pH and/or salinity. Using dual-chamber "H-type" microbial electrolysis cells with graphite rod anodes poised at +0.07 V vs. SHE, we evaluated strains belonging to the genus Geoalkalibacter in the family Geobacteraceae for anode respiration capabilities under either saline (20 g/l NaCl) or alkaline (pH 9-10) conditions. We achieved current densities in the range of 2-7 A m⁻² and visibly thick anode biofilms for two Geoalkalibacter strains when we fed 20 mM acetate as the sole electron donor. Low scan rate cyclic voltammetry (CV) revealed Nernst-Monod anode respiration kinetics and maximal current densities that were not shared across strains, suggesting differences in extracellular electron transfer mechanisms. Comparison of CVs with those of axenic biofilms of G. sulfurreducens indicated saturation of catalytic current waves at similar potentials (+0.05 V), but half-saturation potentials were shifted ~60 mV more negative for Geoalkalibacter grown under alkaline conditions. Further electrochemical, microbiological, and physical characterizations will elucidate the biochemical strategies used for extracellular electron transfer under saline and/or alkaline conditions, as well as reinforce the practicality of Geoalkalibacter and other halophilic and alkaliphilic genera for electricity generation in MXCs.

The brown algae as the electron donor in Microbial Fuel Cells

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The present work examines microalgae as the organic substrate in a two-compartment microbial fuel cell for electricity generation. The brown algae was used as a model substrate for macroalgae, as it is a prolific organism on the earth whose growth requirements are often met by under-utilized ocean resources. The study was conducted under fed-batch mode using mixed microbial community as the biocatalyst and Laminaria saccharina as the electron donor in the anode compartment, and ferricyanide as the electron acceptor in the cathode compartment. The L. Saccharina was processed with two different extraction techniques to aid in the release of chemical oxygen demand during initial phases of MFC operation. The following three test MFCs were obtained by treating sun-dried L.saccharina processed with different extraction techniques: cooked at 131°C, 1.5 atmospheres and for 20 minutes (Auto), exposed to microwave irradiation for 20 minutes (Micro), and without any treatment (No-Treat). A control MFC was initiated with glucose as the sole source of COD (Control1). The defined culture medium containing glucose was used as the electrolyte to promote biofilm formation in all the four MFCs during first two cycles of operation lasting for nearly 500 hours. The test MFCs with biofilm-coated anode were used to evaluate the Kelp for electricity production for additional 400 hours during last two cycles of MFC operation. The four MFCs (Auto, Micro, No-Treat, and Control1) produced power over 100 mW/m². AC impedance techniques were used to identify the major sources of internal resistances in algae-based MFCs.

Alternate Charging-Discharging of CO2-Consuming Biocathodes Derived from Methanogen-**Rich Sediment**

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Electromethanogenic biocathodes produce methane through the reduction of CO2 using electrical current, providing an alternative approach for storing excess renewable energy. A deeper understanding of these electrotrophic communities and their interactions with electrodes is needed before full-scale models are realized. Three diverse innocula known to be rich in methanogenic organisms were added to separate single-chamber microbial electrolysis cells (MECs) and operated for several months using an applied whole-cell potential of 0.7 V. All established biocathodes exhibited a substantial decrease in cathodic overpotential and charge- transfer resistance compared to sterile controls. After polarization (charging) of the bog-derived biocathodes, initial cyclic voltammetry (CV) scans (discharging) revealed a large oxidative peak with a midpoint potential of roughly -450 mV (vs SHE), which was absent in all other cultures. Alternate charging-discharging of the bog biocathode was reproducible, with consistent disappearance of the peak during the second cycle of CV scans. Continuous reactor sparging with either a N2/CO2 or H2/CO2 mix (80%/20% for both) did not affect the charge-discharge cycling, suggesting that H2 did not serve as an intermediate. Varying the length of polarization (0.5 – 5 hrs) led to a shift in the number of oxidative peaks present, their location, and height. This observed charging-discharging effect may be due to electron storage within redox active centers (capacitance) or accumulation of internal storage polymers which are subsequently oxidized during anodic voltammetric sweeps.

Bench Scaled Voltage Enhanced Stacked Microbial Electrolysis and Desalination Cell with **Electrolyte Recycle**

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A microbial desalination cell (MDC) uses exoelectrogenic bacteria to oxidize organic matter, producing a current which can be used to desalinate water placed between alternating ion exchange membranes. Studies have focused on lab scale reactors with electrode chamber volumes around 30 mL. For this MDC study, a multi-anode, bench-scale stacked MDC was used that had an anode chamber volume of 160 mL, with continuous parallel flow concentrate and diluate streams. Using an initial NaCl concentration of 35 g/L, preliminary results have shown a 95.6±1.8% reduction in conductivity of the same saline sample after a total of four 48 hour batch cycles. In order to increase the desalination rate, a voltage can be applied to the system, increasing current density and producing hydrogen from the cathode, making the reactor a microbial electrolysis and desalination cell (MEDC). In multi-anode systems, the difference in anode potentials creates inefficiencies in current generation. In comparison to applying a voltage, desalination rates are also studied while setting anode potentials to -0.2 V using a potentiostat. Low anode pH has been shown to inhibit exoelectrogenic bacteria activity. In order to help balance anode pH, part of the catholyte was recycled and fed with the anolyte at 50, 100, 150 and 200 mM total NaCl concentrations at 12, 15, 18, 21 hour point intervals during a 24 hour batch cycle. These results provide insights into the function of multi-electrode bioelectrochemical systems, and demonstrate increased desalination rates with a bench scale MDC, which is a vital step towards commercial applications of this technology.

Photoautotrophic growth of Rhodopseudomonas palustris supported by anaerobic iron cycling in a biocathode

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It has previously been proposed that oxidation of Fe(II) could be a potential mechanism for supporting growth of electrochemically active bacteria with negatively poised electrodes. Here, we demonstrate that the abiotic reduction of Fe(III) to Fe(II) at an anaerobic cathode facilitates photoautotrophic growth of *Rhodopseudomonas palustris* DX-1 under neutral conditions with electrons derived from an electrode. This process was observed to be dependent on light, demonstrating that the energetics of this mechanism require an external input of energy to overcome the requirements for CO2 fixation for growth. These results show that iron cycling at biocathodes could be responsible for supporting a portion of the sustained electrochemical activity observed with undefined mixed microbial cathode communities.

Power Enhancement with Carbon Nanotube Grown Macroporous Stainless Steel Mesh Anode in Microbial Fuel Cells

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In microbial fuel cells (MFCs), the anode reaction is one of the major sources of electron loss, hence developing highly efficient anode materials is key to enhancing MFC performance. The most commonly used anode materials are carbon-based and include carbon cloth, carbon paper and carbon felt. In recent years, various nanomaterials such as gold nanoparticles and carbon nanotubes (CNTs) have been used as anode materials to improve the interaction between electricity-generating microorganisms and electrodes through various mechanisms such as increased surface area, higher conductivity, and better stability. However, these nanomaterials are typically attached to the electrode as they have been fabricated separately. Our hypothesis is that CNTs directly grown on electrodes can establish a tighter linkage with the underlying electrodes and produce higher power due to enhanced microbe-electrode coupling. The anode presented here utilizes multi-walled carbon nanotubes (MWCNTs) that are directly synthesized on stainless steel (SS) through chemical vapor deposition. Maximum power density using these electrodes was compared against bare SS mesh and bare carbon cloth. We have used our previously developed 24-well MFC array (MFCA) to screen multiple electrode compositions in parallel. Shewanella oneidensis MR-1 was used as the anode respiring bacteria after incubation in tryptic soy broth, and the cathode chambers were operated with potassium ferricyanide (100mM). The maximum power density of MWCNT-grown SS mesh was 545 mW/m², which was 1360 times higher than bare SS mesh (0.4 mW/m²) and 77 times higher than bare carbon cloth (7 mW/m²). The greater than 1300-fold improvement of MWCNT-grown SS mesh anode is, to our knowledge, the largest improvement of any nanomaterial-decorated anodes. This result shows that electrodes on which nanomaterials are directly synthesized have the potential to produce much higher power than electrodes fabricated using conventional approaches. In addition, the effects of CNT lengths and diameters were compared to understand how these parameters influence MFC performances. Results show that longer and larger MWCNT-grown SS mesh can produce 15-fold higher power than shorter and smaller ones (35 mW/m²). Our future work is directed toward further optimizing the electrodes and understanding how the various properties of CNTs influence MFC performances.

Integration of Microbial Fuel Cell Output Metrics and Nonlinear Modeling Techniques for **Smarter Biosensing**

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Microbial fuel cells (MFCs) are promising tools for water quality monitoring and have been deployed as part of a sensory network. It consists of an anode exposed to an electron donor (e.g. an organic pollutant), a cathode exposed to a terminal electron acceptor (e.g. oxygen), and the two chambers are typically separated by a permeable membrane. Bacteria grow on the anode, oxidizing organic compounds, and producing electrons that are transported exogenously to the electrode. Therefore, the presence of soluble organic pollutants triggers the generation of current that can be measured and correlated with water quality data. However, the response peaks have not been well characterized and the data processing methods require improvement, so there is very little quantitative information about the shape of the response profiles.

In this study, long term performance data for an air-cathode MFC system were collected in the lab and in the field (Old Woman Creek Estuary, OH). We explored manual correlations, and integrated two nonlinear mathematical algorithms, artificial neural networks (ANNs) and time series analysis (TSA) model, into MFC-based biosensing to understand water quality parameters. During laboratory testing, the MFCs generated well-organized normally-distributed peaks when the influent chemical oxygen demand (COD) was 150 mg/L or less, and multi-peak signals when the influent COD was 200 mg/L. The area under the response peak correlated very well with the influent COD concentration. During field testing, we observed normally-distributed and multi-peak profiles at low COD concentrations. The ANN predicted the COD concentration without error with just one layer of hidden neurons, and the TSA model predicted the temporal trends present in properly functioning MFCs and in a device that was gradually failing. This report is the first effort to propose peak area as an appropriate MFCs response metric and to integrate **ANNs** TSA with MFC-based biosensing. and

Subsurface microbial electrochemical systems to elucidate the mechanisms of competition between methanogenesis and microbial iron(III)/humic acid reduction in Arctic peat soils

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High-latitude peat soils are a major carbon reservoir, and there is growing concern that previously dormant carbon from this reservoir could be released to the atmosphere as a result of continued climate change. Microbial processes, including methanogenesis and carbon dioxide production via iron(III) or humic acid reduction, are at the heart of the carbon cycle in Arctic peat soils ¹. The underlying biogeochemistry of high-latitude peat soils is poorly understood, particularly the factors governing interspecies competition. As a result, there are significant uncertainties in greenhouse gas emissions, especially methane, in global climate models ^{2,3}. In previous years, we have demonstrated the viability of a potentiostatically-controlled subsurface microbial electrochemical system-based biosensor that measures microbial respiration via exocellular electron transfer ⁴. This system utilizes a graphite working electrode poised at $0.1~V_{\text{NHE}}$ to mimic ferric iron and humic acid compounds. Microbes that would normally utilize these compounds as electron acceptors donate electrons to the electrode instead. The resulting current is a measure of microbial respiration with the electrode and is recorded with respect to time. Here, we examine the mechanistic relationship between methanogenesis and iron(III)- or humic acid-reduction by using these same microbial-three electrode systems to provide an inexhaustible source of alternate electron acceptor to microbes in these soils. Chamber-based carbon dioxide and methane fluxes were measured from soil collars with and without microbial three-electrode systems over a period of four weeks. Initial results indicate that methane emissions were generally consistent within each soil collar throughout the study period, and varied amongst the collars primarily as a function of fine-scale spatial heterogeneity; meanwhile, carbon dioxide emissions fluctuated daily within each collar based on changes in weather, but these fluctuations were largely consistent between all treatments. The results from this work aim to increase our fundamental understanding of competition between electron acceptors, and will provide valuable data for climate modeling scenarios.

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Improved utilization of the anode in a microbial fuel cell stack Shewanella oneidensis as a model biocatalyst

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A single chambered microbial fuel cell was chosen as a model batch reactor to produce electricity from lactate in presence of riboflavin supplements. Shewanella oneidensis DSP10 served as a biocatalyst, graphite felt as the anode and air breathing Pt/C coated gas diffusion electrode as the cathode. Limit to power maxima $^{\sim}5$ W/m³ is largely set by anode impedance (R_A=168 Ω) followed by ohmic losses (R_{Ohmic} = 33 Ω). Cathode impedance (R_c=10 Ω) played a less-influential role as there was no performance improvement when MFC was modified with dual-air breathing cathode configuration (C2). However, power output has increased by ~40% when MFC architecture in C2 was reconfigured into two individual, yet identical MFCs (C3A & C3B), and stacked in parallel connection (C4P). Improved performance of stack vs. unit cell, of equivalent capacity, emerged from huge reduction in RA i.e. 170 Ω vs 39 Ω . Parallel arrangement of cells in the stack outperformed the series arrangement and likely reasons are discussed here. Along with these results, protein analysis and SEM imaging indicated position dependent, nonhomogenous distribution of biofilm on the anode surface. Evolved biofilm characteristics appeared to be a major contributor to R_A.

Investigating the Functionality of a Photosynthetically Driven Microbial Fuel Cell Utilizing Rhodospirillum rubrum

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This study analyzes the potential viability of the bacterium Rhodospirilum rubrum (a purple non-sulfur photosynthetic bacteria) in producing electricity. It is an abundant bacteria actively used in wastewater treatment and also has not yet been investigated in a microbial fuel cell. A prototype for a photoMFC was developed using clear PVC, a commercially available cation exchange membrane, and carbon cloth and steel electrodes. Initially monitoring growth and potential, results showed that R. rubrum viably produced power in a microbial fuel cell, with peak potential of approximately 920 mV occurring under a fluorescent light. Having established R. rubrum's capacity for fuel cell performance, wavelength of exposed light and resistance were modified to determine optimal conditions. An analysis of variance (ANOVA) revealed that the differences in power outputs under varied wavelengths were statistically significant (p < 0.01); the peak in measured power output occurred under the wavelength λ = 470 nm. Power curves were calculated to determine optimal resistance by means of a quadratic regression analysis ($r^2 = 0.88$, p < 0.05). In addition, the fuel cell's potential was monitored under sunlight (in a greenhouse) over the course of 10 days, with results showing that the photoMFC indeed could perform effectively under practical outdoor conditions. Using optimization analyses, the R. rubrum photoMFC was predicted to produce a maximum instantaneous power of 1.2W/m². In comparison to other high power output photoMFCs such as an R. palustris fuel cell with reported power outputs of 2.72W/m², the R. rubrum photoMFC performed about 44% effectively (Xing, 2008). Further repetitions of these studies are required to build sample size and analyze power output variance between R. rubrum fuel cells.

Increasing energy recoveries from MFC/MEC coupled systems

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A microbial fuel cell (MFC)-microbial electrolysis cell (MEC) system was coupled with a capacitor based energy storage circuit to increase the hydrogen production rates without consuming grid electricity while improving energy recoveries with the MFC/MEC coupled reactors aligned in parallel. Without a capacitance based circuit, arranging MFCs in series could be used to increase voltage to that needed to drive efficient hydrogen evolution in MECs; however, voltage reversal is not preventable for this arrangement. Voltage reversal causes irreversible damage to the biofilm anode as the electrode polarity is reversed by externally connected MFCs. Aligning MFCs in parallel increases the current, but does not increase the voltage. In order to realize the benefits of MFCs arranged in parallel, arrays of capacitors were aligned parallel to the MFCs, and switched to a series configuration when discharging to the MEC achieving higher applied voltages. Through the use of this capacitor based circuit, the voltage from the MFCs aligned in parallel was increased by n times, where n is the number of capacitors. It was found that the optimal capacitance per MFCs needed to increase voltages was ~0.01 Farad:1 MFC. Additionally, energy recoveries with the capacitor circuit were shown to increase by ~66%, and hydrogen production rates increased by as much as 50%. These results enable greater hydrogen production to be obtained from MFC/MEC coupled systems aligned in parallel.

Deterioration of the cathode performance in microbial fuel cells by the precipitation of phosphorus

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Two air-cathode single chamber microbial fuel cell (MFC) systems with a total volume of 375 mL (reactor and recirculation bottle) were operated with synthetic wastewater containing approximately 33 mM phosphate. To one MFC (MFC-add), 7.5 mL of the solution containing both ammonium and magnesium (NH₄Cl: 1000 mM, MgSO₄: 1000mM) was continuously added for 96 hours using a syringe pump. To the other MFC (MFC-ctrl), milli-Q water was added under the same condition (with the addition of neither ammonium nor magnesium).

In MFC-add, approximately 40 % of phosphate was removed from the wastewater by the addition of ammonium and magnesium. On the surface of the liquid side of cathode, a lot of precipitate, mainly composed of struvite was formed. On the other hand, phosphate was not removed, and no precipitate on the cathode was observed in MFC-ctrl.

Before starting the addition, both MFCs showed the similar power generation. However, the power generation of MFC-add was decreased compared to the MFC-ctrl after starting the addition. By the linear sweep voltammetry analysis, it was revealed that the performance of the cathode with precipitate was notably deteriorated compared to the cathode of the control.

The decrease in cathode performance might be because mass transfer near the cathode was blocked by the precipitate to some extent. This indicates that for the removal of phosphorus by MFC together with good power generation, it is important to remove precipitate from the cathode at a proper interval to recover oxygen reduction performance.

Simultaneous Recovery of Electrical Power and Phosphorus from Wastewater by Microbial **Fuel Cell**

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Duplicate air-cathode single chamber microbial fuel cells (MFCs) were operated with swine wastewater. During 49 days operation, both MFCs showed similar performances of electricity generation and COD removal. The average value of the maximum power density, COD removal efficiency and the columbic efficiency was 1.67 W/m², 91 % and 37 %, respectively. Furthermore, approximately 80 % of phosphorus was removed from the influent. After the operation finished, a large amount of precipitation was observed on the surface of liquid side of cathodes. The main component of these precipitates was revealed as struvite (magnesium ammonium phosphate: MAP) by XRD analysis. Moreover, the XRD pattern of the precipitate was different from that of the suspended solid in the influent swine wastewater. These results suggest that phosphorus in swine wastewater was removed from wastewater as a result of precipitation on the cathode surface as struvite.

Then, synthetic wastewater containing phosphate was treated by air-cathode single chamber MFCs. By the addition of ammonium and magnesium, phosphate was removed from the influent. On the surface of the liquid side of cathode, a lot of precipitate was formed. The main component of this precipitate was also revealed as struvite (magnesium ammonium phosphate) by XRD analysis. This result indicates phosphorus in wastewater can be removed and recovered by microbial fuel cell with the addition of ammonium and magnesium.

Catalysts and Electrode Architectures for HER in Ammonium Bicarbonate

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The hydrogen evolution reaction (HER) is the reductive half-reaction of water splitting and is a widely investigated process that has been recently regaining considerable attention in respect to the potential role of hydrogen as an energy carrier. However, state-of-the-art commercial water electrolysis is generally limited to highly alkaline conditions and Ni-based catalysts. It has been shown that sustainable hydrogen can be produced with high efficiency by different types of biodegradable organic matter in microbial electrolysis cells (MECs). In addition, the process can be boosted by salinity-gradient energy derived from reverse electrodialysis (RED) but the economic viability of RED technologies confines them to coastal areas with a steady supply of fresh and sea water. This geographic limitation can be overcome by the use of a thermolytic solution based on ammonium bicarbonate, which can be concentrated and regenerated by a cheap source of energy such as waste heat.

We are investigating how to more efficiently achieve system integration (between the electrode compartments and the RED stack) by using ammonium bicarbonate as the electrolyte for the HER. In this study, we investigated and characterized the electrochemical behavior of different HER catalysts and electrode architectures in ammonium bicarbonate solutions. We have identified electrodes based on MoS₂ and hydrophilic binders as potential candidates for economic and efficient process. It was shown that ammonium bicarbonate had superior properties for HER compared to other electrolytes, which improves the prospects for using it in RED-aided hydrogen production technologies.

Determination of Optimal Cathode Catalysts and Binding Agents for Microbial Desalination Cells

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Catalytic reactions on the cathode are an important component of the MDC process. The efficiency and cost of the cathode catalyst and its preparation play a critical role in determining an MDC's performance and application. The most commonly used catalyst is platinum (Pt), an expensive noble metal. The Pt catalyst can effectively catalyze oxygen reduction and ensure a healthy operation of bioelectrochemical systems but its high cost limits the use in large scale reactors. Reducing the Pt loading rate can greatly decrease the capital investment and the research found that the Pt loading rate as low as 0.1 mg/cm2 could maintain an efficient cathode reaction. Non-Pt catalysts have also been investigated with promising results, but they generally require a complex procedure of preparation. Activated carbon is a simple and readily available compound with a potential as the cathode catalyst. However, it has not been examined how well activated carbon can support the cathode reactions in MDCs. In addition, catalysts are usually applied to the cathode electrode using binding agent, such as Nafion solution, which is costly and can increase the internal resistance of the system. Alternative option is polytetrafluoroethylene (PTFE), which has a much lower cost compared with Nafion, and has been used to create diffusion layers in MFCs. In this study, we investigated the effects of the Pt loading rates, binder agents, and activated carbon (AC) as a cathode catalyst in MDCs. To minimize the influence of (different) anode electrode, multiple cathode electrodes were linked to the same anode (and the salt chamber). Electric current was used as a parameter to evaluate the MDC performance.

Experiences in Running a Demonstration Project for More Than One year of a 2-Liter MFC Powering a Fan

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A larger-scale MFC reactor with an internal volume of 2.0 L was operated for more than one year in fedbatch mode. The purpose of the project was to show electricity generation by running a small fan that had the equivalent load of 13 ohms. The anode was (4) graphite fiber brush anodes made using titanium wire that were connected together. Two cathodes made of activated carbon on a stainless steel mesh support were connected to form a single electrode. Acetic acid was used as the substrate for microbial growth. The maximum current density based on polarization data was 0.975 A/m² and the maximum power density was 161 mW/m². Performance fluctuated noticeably over the 12 month period, with the fan initially running constantly, but later only operating for short periods of time. The methods used to try to increase power generation and keep the fan running included reinoculation several times from other working MFC's, different feeding strategies, and replacement of the cathodes. The successes and failures of this demonstration project will be discussed in this poster.

The Fate and Effects of Nitrate and Sulfate on Power Generation in Microbial Fuel Cells

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Microbial fuel cell (MFC) is an innovative bio-technology capable of degrading organic compounds from wastewater to simultaneously achieve water treatment and electrical energy generation. The driving force for the power generation process in MFCs is extracellular electron transport from the anode to the cathode conducted by anaerobic exoelectrogenic bacteria. Besides oxygen, nitrate (NO₃-) and sulfate (SO₄²⁻) are among the most predominant electron acceptors found in wastewater systems where MFCs may be applied. This study investigated the fate and effect of nitrate and sulfate present in wastewater on the performance of single chamber MFC (SCMFC) systems, in terms of voltage, organics degradation and electrochemical characteristics. Plain carbon cloth was used as anode and the non-platinized (Ptfree) carbon cloth was used as cathode in SCMFCs. The study would further elucidate the changes in the anaerobic microbiological communities due to nitrate and sulfate at the anode and cathode electrodes.

Preliminary results indicated that the SCMFCs performance was proportional to the typical concentrations of nitrate (10-30 mg/L) and sulfate (20-60 mg/L) tested, with higher concentrations increasing the cell voltage, power generation [peak power (NO₃: 65.40±6.58mW/cm²); (SO₄²: 83.10 \pm 5.0mW/cm²)] and organics degradation [Δ COD (NO₃-:71 \pm 2%);(SO4₂-:76 \pm 4%);], versus un-spiked control samples [(peak power: 40.0±8.5mW/cm²); (\(\Delta \text{COD:} 68±6\))]. Cyclic Voltammetry (CV) of the systems indicated the presence of H₂S in both nitrate and sulfate spiked SCMFCs, suggesting that the nitrate reduction cycle was associated with sulfate reduction. Linear Sweep Voltammetry (LSV) on the electrodes indicated that SCMFC systems spiked with higher amount of sulfate and nitrate had better performance over the control systems, but the sulfate-spiked SCMFCs better performed over nitratespiked systems, possibly due to the potential inhibition of the electrogenic bacteria at the anode. These data suggests SCMFCs with biocathodes (Pt-free), and specifically sulfate reducing bacteria (SRB), appear to mediate power generation through metabolic pathways, which might encompass cyclic production of sulfur intermediates, and as well oxygen reduction reaction (ORR). The study reveals a great promise of using biofilms as cathode catalyst to reduce the cost of MFCs to treat different contaminants in wastewater, and potentially increase power generation.

Effects of Anodic Carbon Fiber Brush Design in the Electrochemical Performance of Microbial **Fuel Cells**

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Improving microbial fuel cells (MFCs) performance and enabling a cost-effective utility-scale operation require both compact designs and an efficient use of materials. Single chamber cubic MFCs (28 mL), with one or multiple graphite fiber brush anodes and a single air-cathode cathode chamber were used for this study. The effects of titanium wire gauge and fiber length of graphite fiber brushes were examined in duplicate reactors with four different anode configurations. Maximum voltages produced in fed-batch mode, maximum power density, chemical oxygen demand (COD) removal, and coulombic efficiency (CE) were studied to compare the performance of MFCs under these different conditions. The comparison of two wire gauges (1.5 mm and 0.8 mm in diameter) showed no significant difference in MFC performance for the aforementioned parameters, thus evidencing that thinner wires can be equally used in the generation of high power densities, reducing titanium usage and its associated costs by 70%. The fiber length (25 mm, 12 mm and 8 mm in diameter) and use of multiple brushes (one, three and six, respectively) showed some variations among the same factors. Single-brush reactors show a maximum power density of 1,140 mW/m² compared to a maximum of only 1,000 and 830 of the multi-anode systems (3 and 6 brushes, respectively). Whereas the COD removal is almost the same for all configurations (over 90%), the CE is almost 10% higher with single-brush reactors for all external resistances (except for 1,000 Ω). In addition, the projected surface of the anode related to the cathode, as well as the parasitic current generated between adjacent anodes, were determined to have a strong impact on the overall MFC performance of these configurations.

Current generation in microbial electrolysis cells with addition of amorphous ferric hydroxide, Tween 80, or DNA

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Several chemicals added to microbial fuel cells (MFCs), including iron-oxide nanoparticles and the nonionic surfactant Tween 80, have been shown to substantially improve power generation in MFCs. This was thought to occur by improving electron transfer from the bacteria to the anode, although effects on the cathode were not previously addressed. Here, we examined whether amorphous ferric hydroxide [Fe(OH)₃] or Tween 80 could affect current production in single-chamber microbial electrolysis cells (MECs), where hydrogen gas is produced at the cathode, using mixed cultures or a pure culture of Geobacter sulfurreducens. Fe(OH)₃ addition increased the maximum current density of mixed cultures from $6.1 \pm 0.9 \text{ A/m}^2$ to $8.8 \pm 0.3 \text{ A/m}^2$, and from $4.8 \pm 0.5 \text{ A/m}^2$ to $7.4 \pm 1.1 \text{ A/m}^2$ for G. sulfurreducens. These increases were primarily due to improved cathode performance, and improved current production was sustained even after iron was no longer added to the medium. Analysis of the MECs using electrochemical impedance spectroscopy (EIS) showed that the iron reduced primarily the diffusion resistances of the cathode electrodes, and scanning electron microscopy (SEM) images showed the formation of highly porous structures on the cathode. In contrast to previous reports with MFCs, the addition of Tween 80 did not increase current in MECs. Extracellular DNA has been shown to be important for biofilm integrity, and some DNA is electrically conductive, but DNA addition did not improve MEC or MFC performance. These results demonstrated that among these treatments only Fe(OH)₃ addition is a viable method for enhancing current densities in MECs, primarily by improving cathode performance.

Carbon Based Nanomaterials for Micro-sized Microbial Fuel Cells

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Microbial Fuel Cells (MFCs) utilize the naturally occurring power generated by electrogenetic bacteria to produce clean and renewable energy. Microsized MFCs can be used as portable power supplies, on-chip energy sources, or as testing arrays to optimize materials and conditions for implementation into larger scale MFCs. We have developed an assortment of micro-sized MFCs, volumes varying between 1-100 micro-liters, and devices ranging from being directly built onto a silicon chip to flexible mobile platform. A major component of the MFC is the anode which must be both conductive and biocompatible to encourage bacterial growth. Carbon is one of the most frequently used anode materials and hence we have explored carbon based advanced nanomaterials such as MWCNT and graphene to improve performance. Nano-engineered carbon materials have enhanced electrical properties and high surface areas. Our carbon nanotube 1.25 uL cell (Mink et al Nanoletters 2012) produced record current densities and we have also explored the use of graphene in a variety of cell sizes. Because of their rapid testing times (typically 1/6 that of the standard 28 mL cell), microsized fuel cells can be used to test these materials quickly with this knowledge being applied to improve larger scale fuel cells as well. This presentation will be an overview of carbon nanomaterial use in micro-sized microbial fuel cells.

Hydrogen Generation in Microbial Reverse-Electrodialysis Electrolysis Cells Using a **Thermolytic Saline Solution**

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A microbial reverse-electrodialysis electrolysis cell (MREC) can be used to produce hydrogen gas from organic matter and salinity-gradient energy without an external power source. Reverse electrodialysis (RED) is a method for converting salinity differences between high and low concentration solutions into electrical power, and a RED stack can be integrated into a microbial electrolysis cell (MEC) between the electrodes, making a MREC. In order to drive salinity-gradient energy, we used a thermolytic saline solution in the stack, ammonium bicarbonate, which allows for the highly saline solution to be regenerated using low-temperature waste heat ($T \ge 40$ °C). In this study, we examined different salinity ratios (SRs), salt concentrations at a fixed SR, and flow directions (co- or counter-current) of solutions in the RED stack. The maximum hydrogen yield was 3.4 mol H₂/mol acetate (H₂ volume=30 mL), with a hydrogen production rate of 1.6 m³ H₂/m³·d at a salinity ratio of infinite. The highest energy efficiency, based on the consumed energy in the reactor, was 22% and energy recovery, based on total energy applied, remained low (<10%) as a large amount of salinity gradient energy was added to the stack. The catholyte (sodium bicarbonate) concentration affected the cathode overpotential, and the catholyte conductivity should be high to maximize hydrogen production rates. The flow direction of the HC and LC solutions (co- or counter-current) did not affect performance in terms of hydrogen gas volume, production rates, or stack voltages. Thus, the MREC can be successfully operated using ammonium bicarbonate salts that can be regenerated using conventional distillation technologies and low-grade waste heat making the MREC a renewable method for energy production from wastewater.

Pulsed Electric Field as a Pre-Treatment for Enhanced Electron Recovery from Waste **Activated Sludge in Microbial Electrochemical Cells (MXCs)**

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We applied Pulsed Electric Field (PEF) pre-treatment using the Focused Pulsed (FP) technology to enhance bioavailability of Waste Activated Sludge (WAS) for increased electron recovery in a microbial electrolysis cell (MEC) anode. In FP treatment, the WAS is subjected to high-voltage pulsed electric fields for a short time (31 kV for 8 µsec), whereby the sludge flocs and cells are disrupted, making them readily bioavailable for use as an electron donor. Focused Pulsed (FP) treatment of Waste Activated Sludge (WAS) increased the release of soluble COD (11X compared to control) and methane production (1.5X compared to control). Coulombic efficiency in an MEC anode was 1.6-fold higher after FP treatment (33%) compared to control WAS (18%). Likewise, FP-treated WAS sustained a higher maximum current density of 1.4 Am⁻² for a longer duration, while control WAS MEC anode produced a stable current density of 0.3 Am⁻². Methanogenesis accounted for 13% of the total electron consumption in the control WAS, but only 7% for the FP-treated WAS. Thus, FP treatment favorably increased electron recovery as current while methanogenesis, an undesired sink, was suppressed compared to control WAS MEC. Batch biochemical methane potential (BMP) assays, conducted in parallel with the MEC experiments, showed 1.5-fold higher methane production for FP-treated WAS compared to control WAS, and TCOD removals as methane for control and FP-treated WAS were 23 and 34%, respectively. BMP assays established that FP treatment made COD more bioavailable for methanogenesis, while favoring anode respiration when FP treated WAS was added to an MEC anode.

Fouling of Ion Exchange Membranes in Microbial Desalination Cells

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Microbial desalination cells (MDCs) are a newly developed concept that combines wastewater treatment, salt water desalination, and electricity generation in a single device. Membrane fouling is not well understood or investigated, because most studies about MDCs adopt sodium chloride and acetate solutions, which are very different from actual salt water (e.g. seawater) and wastewater that could cause serious membrane fouling. The aim of this work is to investigate how the membrane fouling affects desalination, to understand how serious the membrane fouling could be under a condition of seawater and actual wastewater, and to determine which membrane, CEM or AEM, will be fouled faster. Multiple MDCs were set up with the same design, but were operated for different period of time, 6, 9, 16, 24 weeks (including start up). At the end of each operation, the MDC was disassembled and then we analyzed membrane fouling by measuring membrane resistance and ion transport. We also used SEM, EDS, and FTIR techniques to examine the surface and cross-section of the membranes. The preliminary results showed that the desalination performance did not obviously decrease during the first 16 weeks. The salt removal was mainly affected by the current, which was affected by the organic concentration in wastewater. However, severe fouling on the ion-exchange membranes was observed. After nine weeks, the resistances of AEM and CEM have increased 16.6% and 28.2%, respectively. After sixteen weeks, the resistance of CEM has increased 34.5%. The EDS and FTIR analysis suggested that the fouling on the anode side of the AEM was mainly biofouling and the CEM was fouled by inorganic compounds.

Energetic and Cellular Redox Potential Effects in Geobacter sulfurreducens from Electron **Acceptor Variations**

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Developing a better understanding of how the metabolic processes of Geobacter sulfurreducens respond to electron acceptor variations could provide useful insights for improving bioelectrochemical system (BES) performance. Cofactors indicative of cellular energetics and redox conditions (i.e., ATP, ADP, AMP, and reduced and oxidized NAD and NADP) were analyzed in G. sulfurreducens cultures using fumarate, Fe(III)-citrate, or anodes poised at varying redox potentials as the electron acceptor. Results showed that cellular redox potential given by ratios of reduced to oxidized NAD and NADP were similar (0.088 ±0.022 for NAD and 0.268 ±0.098 for NADP) when growing under all anode potentials tested and with Fe(III)-citrate, which is also thought to be reduced extracellularly. However, both ratios were significantly increased when fumarate was provided as the electron acceptor, with values of 0.331 ±0.094 (NAD) and 1.955 ±0.369 (NADP). Energy potential of the cells, represented by the adenylate energy charge (AEC), was maintained near 0.47 under almost all conditions tested. The reactor poised at 110 mV versus standard hydrogen electrode (SHE) maintained an AEC of 0.63 ± 0.03, which was statistically different from the AEC for the reactor poised at -190 mV SHE (0.47 ± 0.003) and fumaratereducing cultures (0.047 ± 0.02). Anode biofilms demonstrated a significantly higher ATP/ADP ratio relative to cells growing in suspension on fumarate or Fe(III)-citrate. These results indirectly show that metabolic mechanisms regulated by the ratios of reduced to oxidized NAD and NADP are altered significantly between G. sulfurreducens cultures growing with extracellular and intracellular electron acceptors but that energy potential represented by AEC is not significantly altered. However, the ATP/ADP ratio and specific cellular ATP concentration increased during biofilm growth, suggesting a need for increased levels of ATP to maintain the biofilm. Overall, anode potential had little effect on these metabolic ratios, and specific electron acceptors only appear to affect these ratios based on whether they are intra- or extracellular.

Characterization of Metabolism in Electricity-Producing Shewanella Oneidensis MR-1 Using ¹³C-Based Metabolic Flux Analysis

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In microbial fuel cells (MFCs) microbes catalyze the production of electricity from the oxidation of organic substrates. In addition to having several applications, including wastewater treatment, MFCs present an interesting metabolic system: microbes convert chemical energy to external electrical energy by utilizing a solid electrode as the terminal electron acceptor. We have employed ¹³C Metabolic flux analysis in which isotopically-labeled tracers are applied to elucidate fluxes, to investigate the metabolism of a microbe within an MFC. Shewanella oneidensis MR-1 was selected as the model organism because it has the ability to oxidize organic compounds with an electrode serving as the electron acceptor and its metabolic network has been previously described. We present the fluxes through gluconeogenesis, the TCA cycle, the pentose phosphate pathway, and serine metabolism for S. oneidensis MR-1 in an MFC and compare them with the fluxes in systems utilizing other terminal electron acceptors.

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Oxygen scavangers and enhancing electromethanogenesis in microbial electrolysis cells (MEC)

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In methanogenic MECs, carbon dioxide (CO₂) is reduced to methane (CH₄) by microorganisms that are able to use electrical current. This method has the potential to store an excess of renewable energy in energy carriers, rather than as electrons in batteries, as well as to recycle CO2 into the fuel. One concern is that cathodic methanogenic microbial consortia are prone to oxygen inhibition by trace oxygen (O₂)crossover from the anode if water is being split as a source of electrons. Chemical oxygen scavangers can help to maintain anaerobic conditions and therefore improve performance. Here we demonstrate the suitability of 2 mediators and 8 chemical oxygen scavangers: anthrahydroquinone-2,6-disulfonate (AHQDS), resazurine, ascorbic acid (vitamin C), cysteine, iron sulfide, mercaptoethanol, sodium sulfide, sodium dithionite, titanium(III)-citrate and titanium(III)-oxide. We used cyclic voltametry between -0.9 and 0 V (vs. standard hydrogen electrode) in a double chamber system using a typical methanogenic freshwater medium. Titanium(III) and iron sulfide had the lowest redox effect whilethe mediators AHQDS and resazurine showed clear signals within the typical potential of methanogenic MECs. Additional results on the growth of methanogens in MECs will be presented. Our results demonstrate that oxygen scavangers can be used MECs to maintain anaerobic conditions for microbial methanogenesis, and that the performance of these systems can be improved through operational procedures.

Cyanobacteria that can fix bicarbonate in a microbial electrolysis cell (MEC) cathode without

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Cyanobacteria are known as effective CO2 fixing microorganisms. However, light is usually required which makes an application economically difficult. Here we show that cyanobacteria can take up current and fix bicarbonate without light in an MEC reactor. Three double-chamber MEC reactors were preacclimated in the light at a set cathode potential of -611 mV. All reactors contained a waste water mix in the anode chamber. The cathode chamber was inoculated with either a mix of cyanobacteria, cyanobacteria and wastewater, or wastewater only. The mix of cyanobacteria without wastewater consisted of three different species: *Microcystis* sp. isolated from Lake Taihu in China, *Cyanothece* sp. isolated from a microbial fuel cell as well as Anabaena sp. which was obtained from a culture collection. During 2 weeks incubation in the light, peak current in the cyanobacteria-only reactor was approximately 400 mA while without light (3 weeks) the peak current was one order of magnitude lower. At the same time, this reactor removed bicarbonate from the solution most rapidly with and without light. However, bicarbonate removal rates were faster in the experiment with light. Our results indicate that cyanobacteria can be employed for CO₂ fixation without light. Hence, this may be a viable strategy to extract atmospheric CO₂.

Biocomputing in Bioelectrochemical Systems: Synthetic Biology Approaches to **Electrochemically Active Bacteria**

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Biocomputing has gained interest in recent years, partly because of a need for smart biosensors. The majority of bacterial biocomputing research has been focused on Escherichia coli using fluorescent protein as an output signal. However, it would be beneficial to produce bacterial biocomputers with electrical output signals for simpler measurement and integration with traditional computers. A few research groups have already demonstrated simple logic gates based on microbial electrochemical systems, however, these have been limited by underdeveloped genetic systems for electrochemically active bacteria. We propose to develop Shewanella oneidensis as a platform for bacterial biocomputing based on its well-characterized extracellular electron transport pathway (the Mtr pathway).

Biocathode Development Using Acid Mine Drainage Inoculum

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The exploration of acid mine drainage (AMD) for lithotrophic bacteria able to use an external electron donor (e.g. Fe, H_{2(g)}) and oxygen as electron acceptor at low pH presents a bioprospecting opportunity to develop low-cost biocathodes with acidotolerant or acidophilic exoelectrotrophs.

Three different sediment microbial fuel cell (SMFC) configurations were used to enrich microbial communities from AMD-affected sediments on aerobic graphite fiber brush cathodes: (i) AMD-Winogradsky columns using small (2.5-cm length) brushes (tested for 10 months), (ii) AMD-Winogradsky columns using large (7-cm length) brushes (tested for 1 month), and (iii) AMD-SMFCs inoculated from Winogradsky columns (tested for 3 months). Polarization and power density curves show that the power produced by these Winogradsky columns and AMD-SMFCs are in the neighborhood of published SMFCs constructed with the same electrode material.

Linear sweep voltammetry conducted on these graphite fiber brushes, tested as cathodes in the AMD columns or the SMFCs in 0.08 M Fe(II) aerobic medium at pH 2.6 and conductivity of 27 mS/cm, confirmed improvements in cathode performance due to microbial activity. After gamma irradiation (from 25 to 75 kGy), biocathode performance decreased to the level of abiotic controls.. Potentiostatic electrochemical impedance spectroscopy of AMD biocathodes at -0.45 V showed decreases in charge transfer due to microbial activity. Microbial catalysis was quantified by a decrease in charge transfer resistance (Rc) on AMD biocathodes (Rc $_{biocathode}$ = 5.5 \pm 1.1 Ω) relative to the abiotic control (Rc $_{abiotic\ control}$ = 33.0 \pm 3.21 Ω) and the biocathode after gamma irradiation (Rc biocathode after v-irradiation = 32.8 \pm 6.41 Ω). Observations of the cathode through epifluorescence microscopy showed biofilm formation around the graphite fibers. DNA samples were collected from the fibers, and pyrosequencing is being performed to allow a characterization of the AMD cathodic microbial biofilms.

Key Aromatic Amino Acids Required for Conduction Along Pili of Geobacter sulfurreducens

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The mechanisms for long-range extracellular electron transport in Geobacter species are of interest because of their unique ecological roles and biotechnological applications. Geobacter species are the most important dissimilatory Fe(III) reducers in a diversity of sedimentary environments. They also form syntrophic associations with methanogenic organisms in anaerobic wastewater digesters, exchanging electrons via direct interspecies electron transfer (DIET). Furthermore, Geobacter species are more effective than any other known microorganisms in converting organic compounds to electricity in microbial fuel cells.

The pili of Geobacter sulfurreducens are conductive along their length via a metallic-like conductivity, similar to that observed in synthetic organic conducting polymers. Metallic-like conductivity in a biological protein represents a paradigm shift because previously described mechanisms for biological electron transport rely on electron hopping/tunneling between discrete, redox-active molecules.

The metallic-like conductivity of pili has implications for several forms of long-range electron transport. For example, the c-type cytochrome, OmcS, which is specifically localized on pili, is required for Fe(III) oxide reduction by G. sulfurreducens. Thus, the simplest model for Fe(III) oxide reduction is electron transfer along pili to OmcS, which performs the terminal electron transfer to Fe(III) oxide. The metalliclike conductivity of current-producing biofilms can be attributed to networks of conductive pili, with final electron transfer from the conductive biofilm to an anode catalyzed by the c-type cytochrome, OmcZ. In a similar manner, DIET relies on conductive pili and associated cytochromes.

X-ray diffraction analysis revealed overlapping pi orbitals in pili, consistent with the known role of pi stacking in the metallic-like conductivity of synthetic conducting polymers, such as polyaniline. In order to determine if aromatic amino acids exposed on the outer surface of the pili might contribute to conductivity, we genetically constructed mutant strains in which alanine was substituted for aromatic amino acids located at the carboxyl terminus of PilA, the structural pilin protein. Key aromatic amino acids were identified that are required for electron transfer to Fe (III) oxides and long-range electron transport through biofilms. These results are consistent with the metallic-like conductivity hypothesis, but inconsistent with the alternative model of electron transport along pili mediated by cytochromes.

Synergistic Linkage between Osmotic Microbial Fuel Cells and Microbial Desalination Cells for **Enhancing Seawater Desalination**

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In the present study, an osmotic microbial fuel cell (OsMFC) and a microbial desalination cell (MDC) are hydraulically connected to boost the desalination performance. Acetate solution and artificial seawater is fed into the anode chamber and salt chamber of an OsMFC, respectively. Due to the different in chemical potential, water in acetate solution will migrate from the anode chamber into the cathode chamber. In this way, seawater acts as a draw solution in osmotic process. The anode effluent and cathode effluent of the OsMFC are then used as the anode feed and the salt feed to an MDC to accomplish salt removal. Preliminary results show that by connecting these two systems together, the final seawater effluent from the MDC has a conductivity of 5 mS/cm, much lower than that (28 mS/cm) of an MFC without the OsMFC pretreatment (at the same salt solution HRT). The main reason for the improvement is that OsMFC could draw low conductivity water from its anode and thus significantly dilute the seawater. The combined system shows that it outcompeted stand-alone MDC in reducing the conductivity of the seawater at the same flow rate, which translates to higher effienciency and more economical competence. We are currently working to link the OsMFC to an MEDC, which utilizes the electric potential generated from the OsMFC to produce hydrogen gas.

Simultaneous nitrification and denitrification with electricity generation in dual-cathode microbial fuel cells

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This study presented a dual-cathode microbial fuel cell (MFC) that was designed to accomplish nitrification in its outer cathode and denitrification in the inner cathode. The MFC was continuously operated for more than 150 days and achieved organic removal of 85-96% in the anode, depending on the initial organic loading rates. More than 96% of the ammonium was removed, while the total nitrogen removal was between 66.7 and 89.6%, largely affected by the remaining nitrate in the effluent of the inner cathode. The coulombic efficiency suggested that the nitrate was primarily removed by bioelectrochemcial denitrification in the inner cathode, especially at the low nitrogen loading rates. However, a higher nitrogen loading rate encouraged nitrate migration through the anion exchange membrane, thereby being removed by conventional denitrification. Those results demonstrated the feasibility of using a tubular dual-cathode MFC to remove both organics and nitrogen while producing electricity.

Source of Baseline Current in Microbial Electrolysis Cells

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Batch-operated single-chamber microbial electrolysis cells (MECs) often show a significant baseline current, even when the primary exogenous electron donor is consumed. This makes it difficult to account for electron allocation to current, biomass synthesis, alternate electron acceptor reduction, or other losses. We are studying the factors that cause this baseline current, which is important for understanding the mechanisms of MECs. In this work, two bacterial cultures were used in singlechamber MECs: Geobacter metallireducens, which reportedly does not directly utilize hydrogen for current production, and a mixed culture. Each culture type was fed with sodium acetate or hydrogen and CO2. We found that baseline current occurred only in MECs with Geobacter, but was not observed with the mixed culture. The value of baseline current for Geobacter reached 0.15 mA and lasted for over 3 days without new media. Acetate was detected at low concentrations in the acetate-fed Geobacter metallireducens system after extended baseline current, perhaps indicating a contribution of acetogenic activity at the cathode sustaining this baseline current. In order to separate influence of cathode reactions, experiments are presently being conducted in two-chamber batch-fed MECs.

Improving Startup Performance with Carbon Mesh Anodes in Separator Electrode Assembly **Microbial Fuel Cells**

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In a separator electrode assembly microbial fuel cell (MFC), oxygen crossover from the cathode raises the anode potential and inhibits current generation by exoelectrogenic bacteria, resulting in difficulties in reactor startup and stable performance. In order to improve startup performance, MFCs with carbon mesh anodes were acclimated at set potentials (-0.2 V or +0.2 V versus a standard hydrogen electrode), or with no potential control, and inoculated with wastewater. Performance was compared to reactors inoculated with cell suspensions from existing MFCs under the same conditions. Anodes inoculated with wastewater and acclimated to -0.2 V produced the highest power (1330±60 mW m⁻²) but they had the longest startup time (20 days). The most consistent and reproducible results in terms of reduced startup time (10 days) and high power production were obtained with pre-acclimated cell suspensions. Additional electrochemical analyses confirmed that inoculation with a transferred culture consistently improved anode performance, with the best activity obtained for anodes acclimated at -0.2 V. These results imply that rapid startup of larger-scale reactors will require use of pre-acclimated cultures, and that acclimation at -0.2 V could improve power production compared to a more positive potential (+0.2 V) or a lack of set potential.

Using separator in microbial fuel cells shortens start-up time and improves anode performances at ambient temperature

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Microbial fuel cells (MFCs) are a promising technology for treating wastewater while simultaneously producing clean energy as electricity. Recent studies demonstrated that at mesophilic temperatures the MFCs had shorter start-up time to reach reproducible voltage generation, and also obtained better anode performances, compared to that at ambient temperatures. However, for practical application, the MFC operation is normally conducted at ambient temperature, relatively lower. In this study, it was found that using a glass fiber separator shortened the start-up time and improved the anode performances at ambient temperature (20 ± 3 °C) and mesophilic (30 ± 1 °C) temperatures. At 20 °C, the MFC using a glass fiber separator produced a voltage of 350 mV after 3 d and reached a reproducible voltage generation under 1000 Ω after 8 d, while the MFC lacking a separator produced < 50 mV up to 6 d and obtained start-up time of 10 d. After 30 d operation, the anode in the MFC with a separator achieved a lower potential in polarization tests than that of the MFC without a separator, which also exhibited power overshoot problem at 20 °C (a doubling back of the power curve, indicating that the anodic biofilm was not sufficiently acclimated to higher current densities). The anodes with a separator obtained charge transfer resistances of 18.7 Ω at 20 $^{\circ}$ C and 16.4 Ω at 30 $^{\circ}$ C, which were 76% (20 $^{\circ}$ C) and 19% (30 °C) lower than that of the MFCs without a separator. The coulombic efficiencies (CE) of MFCs with a separator were 89% (20 °C) and 87% (30 °C) at the current density of 6.7 A/m², which was significantly higher than that of MFCs without a separator at both 20 °C and 30 °C (16%~43%). The main function of separator was to prevent oxygen intrusion and thus to provide the low dissolved oxygen concentrations needed for electricity production by bacteria. These results demonstrated that using glass fiber separators in MFCs shortened start-up time and enhanced the anode performances.

Set Potential Regulation Reveals Additional Oxidation Peaks of Geobacter sulfurreducens **Anodic Biofilms**

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Higher current densities produced in microbial fuel cells and other bioelectrochemical systems are associated with the presence of various Geobacter species. A number of electron transfer components are involved in extracellular electron transfer by the model exoelectrogen, Geobacter sulfurreducens. It has previously been shown that 5 main oxidation peaks can be identified in cyclic voltammetry scans. It is shown here that 7 separate oxidation peaks emerged over relatively long periods of time when a larger range of set potentials was used to acclimate electroactive biofilms. The potentials of oxidation peaks obtained with G. sulfurreducens biofilms acclimated at 0.60 V (vs. Ag/AgCl) were different from those that developed at -0.46 V, and both of their peaks were different from those obtained for biofilms incubated at -0.30 V, 0 V, and 0.30 V. These results expand the known range of potentials for which G. sulfurreducens produces identifiable oxidation peaks that could be important for extracellular electron transfer.

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NA-ISMET Abstract Book

May 13-15 2014

THE PENNSYLVANIA STATE UNIVERSITY

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KEYNOTE LECTURERS

We are pleased to have several distinguished professors speaking at this conference.

Mike Hickner, Department of Materials Science and Engineering, Penn State University

Derek Lovley, Department of Microbiology, University of Massachusetts

Korneel Rabaey, Laboratory of Microbial Ecology and Technology (LabMET), Ghent University

César Torres, School for Engineering of Matter, Transport and Energy, Arizona State University

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Lars Angenent, Department of Biological and Environmental Engineering, Cornell University

Gui Bazan, Department of Chemistry and Biochemistry, University of California Santa Barbara

Mohammed El-Naggar, Department of Physics and Astronomy, University of Southern California

Yujie Feng, State Key Laboratory of Urban Water Resource & Environment, Harbin Institute of Technology

Xia Huang, School of Environment, Tsinghua University

Alfred Spormann, Department of Civil & Environmental Engineering, Stanford University

SYMPOSIUM SCHEDULE

| Tuesday, May 13 th , 2014 | | | |
|--------------------------------------|--|---|--|
| 10:30 am- 1:30 pm | Lab Tours 128 Sackett Building | | |
| 1:30-3:30 pm | Workshop (Electrochemical and spectroscopic analysis of METs) 135 Reber Building | | |
| 6:00-8:00 pm | Opening Reception Robb Hall – Hintz Family Alumni Center | | |
| ' | 1 3 1 | , | |
| Wednesday, May 14th | , 2014 Berg Auditoriu | ım – 101 Life Sciences Building | |
| 9:00-9:10 am | Opening comments/introductions | Bruce Logan - Penn State University | |
| Session 1 | Electron Transfer/Biofilms | | |
| 9:10-:9:40 am | Keynote speaker- Electron and ionic transport in microbial anode respiration: fundamental knowledge that leads to applications in microbial electrochemical cells | César Torres - Arizona State University | |
| 9:40-10:00 | Invited Speaker - Hydrogenase- independent uptake and metabolism of electrons by the archaeon Methanococcus maripaludis | Alfred Spormann- Stanford University | |
| 10:00-10:20 am | Invited Speaker- Conjugated oligoelectroytes for bioelectronics applications | Gui Bazan - University of California Santa Barbara | |
| 10:20-10:35 am | Break | | |
| 10:35-10:55 am | Invited speaker - The biophysical and structural basis of extracellular charge transport in microbial redox chains | Mohammed El-Naggar - University of Southern California | |
| 10:55-11:15 am | Electrochemical mechanisms of accelerated anodic respiration by Shewanella oneidensis MR-1 chemically modified with a membrane-intercalating conjugated oligoelectrolyte | Nathan Kirchhofer - University of California, Santa Barbara | |
| 11:15-11:35 am | Effects of additional electron acceptors in bio-anodic processes: oxygen and sulfate | Shiue-Lin Li- University of Southern California | |
| 11:35-11:55 am | Metabolic network in electrogenic biofilms identified by metatranscriptomic approach | Shun'ichi Ishi - J. Craig Venter Institute | |
| 11:55-1:00 pm | Lunch | | |

| Session 2 | Novel microbial ecologies and applications | |
|---------------|--|--|
| 1:00-1:30 pm | Keynote speaker - Surface chemistry of electrodes and its role in microbial adherence and current generation | Korneel Rabaey - Ghent University |
| 1:30-1:50 pm | Conversion of lignin to electric current with a defined co-culture of <i>Rhodopseudomonas</i> palustris and <i>Geobacter sulfurreducens</i> | Devin Doud - Cornell University |
| 1:50-2:10 pm | Produced water treatment capability of lab and pilot scale microbial capacitive desalination systems | Casey Forrestal - University of Colorado, Boulder |
| 2:10-2:30 pm | Improving coulombic recovery in fermentable substrate fed MECs by shifting the products of fermentation | Joseph Miceli - Arizona State University |
| 2:30-2:50 pm | Break | |
| 2:50-3:10 pm | Invited speaker - Enterobacter aerogenes and Pseudomonas aeruginosa at anodes: who's in charge? | Lars Angenent - Cornell University |
| 3:10-3:30 pm | Characterization of the simultaneous fermentation and anode respiration capabilities of the thermophilic <i>Thermoanaerobacter pseudethanolicus</i> in a Microbial Electrolysis Cell (MEC) | Prathap Parameswaran - Arizona State University |
| 3:30-3:50 pm | Cathodes of microbial electrolysis cells (MEC) support preferential growth of methanogens | Michael Siegert - Penn State University |
| 3:50-4:10 pm | Developing microbial electrochemical systems for electricity and hydrogen production in biorefinery | Abhijeet Borole - University of Tennessee |
| 4:10-4:30 pm | Systems biology approach to characterize multispecies biocathode biofilms | Sarah Glaven - Naval Research Laboratory |
| 4:30-5:00 pm | Break | |
| 5:00-6:30 pm | Poster session Fac | culty Staff Club, Nittany Lion Inn |
| 6:30-10:00 pm | Banquet | Boardroom, Nittany Lion Inn |

| Thursday, May 15 201 | 14 | 102 Thomas Building |
|----------------------|--|---|
| Session 3 | Electron Transfer/Biofilms | |
| 9:00-9:30 am | Keynote speaker - Synthetic electromicrobiology | Derek Lovley - University of Massachusetts, Amherst |
| 9:30-9:50 am | Continuous production of an oily stream of medium-chain carboxylic acids with a microbial electrochemical technology | Jiajie Xu - Cornell University |
| 9:50-10:10 am | Molecular characterization of the interactions that mediate electron transfer across the microbe-electrode interface in Shewanella oneidensis MR-1 | Catarina Paquete - Universidade Nova de Lisboa |
| 10:10-10:30 am | Metabolic characterization of anode- respiring <i>Escherichia coli</i> | Michaela TerAvest - California Institute for Quantitative Biosciences |
| 10:30-10:45 am | Break | |
| Session 4 | Design and scale-up advancement | |
| 10:45-11:05 am | Invited speaker - Scaling up biocathode microbial fuel cells | Xia Huang - Tsinghua University |
| 11:05-11:25 am | Combined bioelectrochemical-electrical model of a microbial fuel cell | Didac Recio Garrido - École Polytechnique Montréal |
| 11:25-11:45 am | Harvesting microbial fuel cell ionic currents for enhanced energy generation through capacitive mixing | Marta Hatzell - Penn State University |
| 11:45 am - 12:05 pm | Effect of physical and geometrical properties of carbon nanotube anodes on microbial fuel cells | Arum Han - Texas A&M University |
| 12:05-1:00 pm | Lunch | |
| 1:00-1:30 pm | Keynote speaker - Designing ion exchange membranes for new energy processes | Mike Hickner - Penn State University |
| 1:30-1:50 pm | Stable and high voltage and power output of microbial fuel cells internally connected in series | Yanzhen Fan - Oregon State University |
| 1:50-2:10 pm | High performance monolithic power management IC chip system with dynamic maximum power extraction algorithm for microbial fuel cells | Celal Erbay - Texas A&M University |
| 2:10-2:30 pm | High performance of spirally-wound microbial fuel cell design and its analysis using interdisciplinary methods | Alexander Haeger - University of Colorado, Boulder |
| 2:30-2:45 pm | Break | |

| 2:45-3:05 pm | Invited speaker | Yujie Feng - Harbin Institute of Technology |
|--------------|--|---|
| 3:05-3:25 pm | Challenges in controlling methanogens in microbial electrolysis cells | Younggy Kim - McMaster University |
| 3:25-3:45 pm | Nano/micro/macro bioelectronics platform utilizing for scale-up of bio-electrochemical cell | In Seop Chang - Gwangju Institute of Science and Technology |
| 3:45-4:05 pm | Examination of spatial characteristics and gap-bridging ability of conductive mixed species biofilms | Cheng Li - Oregon State University |
| | | |
| 4:05-4:20 pm | Presentation of awards/Closing remarks | Jay Regan - Penn State University |

KEYNOTE PRESENTATION ABSTRACTS

Designing ion exchange membranes for new energy processes

Michael A. Hickner

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Polymeric membranes are an important component in many microbial electrochemical cell (MEC) designs. Polymers serve as anode-cathode separators as well as perform vital permselectivity functions in unit operations such as reverse electrodialysis (RED) stacks or other separation processes. These new types of energy technologies require a re-evaluation of membrane properties and design of new polymers and membrane systems that have low ionic resistance, yet maintain the selective transport required for a specific application in the unique environment of MECs. We have investigated polymer membranes as cathode binders and coatings to decrease cathode fouling and improve the coulombic efficiency of microbial fuel cells (MFC). Additionally, we are optimizing ion exchange membrane compositions to improve RED performance and to understand membrane transport in different electrolytes such as sodium chloride and ammonium bicarbonate. This talk will focus on the critical issues of ion exchange polymer design for MFCs and discuss membrane design details for ammonium bicarbonate-based RED. Emphasis will be placed on how the polymer chemical structure influences transport and key design considerations for deploying polymers in MEC technology.

Synthetic electromicrobiology

Derek R. Lovley

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Some applications of microbe-electrode interactions, such as harvesting current from aquatic sediments and wastewater, are readily accomplished with native microorganisms or mixed microbial communities. However, there are many emerging microbe-electrode applications that can be best realized with synthetically designed microorganisms that are optimized for those applications. Rational design requires a mechanistic understanding. For example, it was feasible to engineer strains of *Geobacter sulfurreducens* to produce higher current densities once the importance of conductive pili in long-range electron transport was recognized. Effective electrosynthesis of valuable organic commodities from carbon dioxide required redirecting the carbon and electron flow away from the production of acetate and ethanol and toward the production of desired commodities. In a similar manner, principles of synthetic biology are now guiding the design of novel biological computational devices that exploit the ability of microorganisms to accept electrical inputs and generate electrical outputs, as well as the development sensors for important environmental parameters.

Surface chemistry of electrodes and its role in microbial adherence and current generation

Kun Guo^{1,2} and Korneel Rabaey^{1,2}

When starting up a microbial bioelectrochemical system, microorganisms need to migrate towards an electrode, adhere and subsequently establish some form of electrical link with it in order to establish a bio-electrode. A multitude of factors including system geometry, nature of the microorganisms, flow regime, applied electrical field and properties of the electrodes impact this sequence towards successful microbial electrocatalysis. In this presentation we will summarize some of the key findings of our and other groups regarding the impact of electrode properties on the process, and how one can tune surface chemistry to accelerate – or avoid – microbial colonization of the electrode. We will also consider how surface modification can be achieved not only for laboratory scale reactors, but also for scalable systems.

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Electron and ionic transport in microbial anode respiration: fundamental knowledge that leads to applications in microbial electrochemical cells

César I Torres

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Anode-respiring bacteria (ARB) catalyze the complete oxidation of organic compounds (e.g. acetate, glucose) into electrical current and carbon dioxide. ARB produces a biofilm at the electrode surface, where even cells on the outer part of the biofilm are participating in current production. Our team uses a variety of electrochemical techniques in order to characterize electron transport responses from various ARB. Through these experiments, we have observed a complex response to anode potential that allows ARB, such as *G. sulfurreducens*, to optimize their efficiency in electron transport. This complex behavior allows us to better understand and predict the response of ARB to different conditions in microbial electrochemical cells (MXCs).

While the topic of electron transport is the focus of most ARB research, ionic transport is the most important factor in determining rate-limiting and potential loss processes. ARB require near-neutral pH in the medium to grow, differing from chemical fuel cells commonly employed, which run under acidic or alkaline conditions. This pH requirement results in a major transport limitation, as H^{+} ions (now in μ M range) should be transported from anode to cathode to achieve electron neutrality. In an MXC anode, H^{+} ions accumulate in the ARB biofilm, creating an acidification that limits current generation. At the cathode, local gradients leading to pH > 12 is typical in MXC operation; as a consequence, the pH gradient results in Nernstian concentration overpotential of > 300 mV. Thus, understanding and controlling ionic transport in MXCs is essential to ensure an efficient operation. I will discuss our current efforts to characterize and overcome ionic transport limitations in order to develop efficient MXC processes.

Invited Presentation Abstracts

Enterobacter aerogenes and Pseudomonas aeruginosa at anodes: who's in charge?

Arvind Venkataraman¹, Yaping Zhang¹, Devin F. R. Doud, Ashim K. Datta, Miriam A. Rosenbaum, and <u>Largus T. Angenent</u>

Department of Biological and Environmental Engineering, Cornell University, USA; <u>la249@cornell.edu</u>

¹These authors contributed equally

We have observed in a potentiostatically-controlled 3-electrode bioelectrochemical system with defined mixed cultures consisting of *Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Geobacter sulfurreducens,* that synergistic mediated electron transfer (MET) by *P. aeruginosa* and *E. aerogenes* was responsible for 56.9 ± 10.1% of the total current production, while direct electron transfer (DET) by *G. sulfurreducens* accounted for the rest. This was the result of cooperative (*i.e.*, between *P. aeruginosa* and *E. aerogenes*) and conflictive (*i.e.*, between *P. aeruginosa* alone contributed only 5.65 ± 1.67% of the total current. We then developed and tested a mathematical model to describe the synergism between *P. aeruginosa* and *E. aerogenes* that made MET an important component of the extracellular electron transfer in our system. In the microbial community, *E. aerogenes* stimulates *P. aeruginosa* to produce more phenazines, resulting in improved MET compared to a pure culture of *P. aeruginosa*. After running the mathematical model, we hypothesized that the re-uptake and rereduction of oxidized phenazines occurred primarily through the activity of *E. aerogenes* rather than *P. aeruginosa*. Consecutive testing on the bench confirmed this hypothesis to be correct. This leaves us with the question: who is in charge at the anode?

Conjugated oligoelectrolytes for bioelectronic applications

<u>Guillermo C. Bazan</u>¹, Logan Garner¹, Alex Thomas¹, Huijie Hou^{1,4}, Jenny Du¹, Nate Kirchhoffer¹, James Sumner², Derek Lovley³, Bruce Logan⁴

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Conjugated polyelectrolytes (CPEs) and conjugated oligoelectrolytes (COEs) are defined by a pi-delocalized backbone and pendant groups bearing ionic functionalities. The ionic component makes these materials unique, in that they combine the optoelectronic properties of organic semiconducting materials with the characteristics of polyelectrolytes. The pi-conjugated framework retains a propensity for incorporation into hydrophobic environments. Cationic COEs based on oligophenylenvinylene repeat units therefore have a tendency to accumulate within the lipid bilayer membranes of artificial vesicles and/or living microorganisms. A trivial application is that of fluorescence imaging. More interestingly, the incorporation of the COE into membranes allows for the fabrication of more efficient microbial fuel cells based on yeast or *E. coli*. Similarly, it has been shown that COEs improves the ability of *S. oneidensis* to use a graphite electrode as the sole electron donor for the reduction of fumarate. A set of mechanistic studies will be discussed that shed light into the possible mode by which bioloelectrochemical function is modified. Finally, we conclude with the observation that the addition of COEs to microbial electrolysis cells (MECs) improves hydrogen recovery, which indicates function beyond that of simple electron shuttles.

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The biophysical and structural basis of extracellular charge transport in bacterial nanowires

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Reduction-oxidation (redox) reactions and electron transport are essential to the energy conversion pathways of living cells. But what if there was also a direct link allowing electron exchange between the living and non-living worlds? We now know that some anaerobic bacteria gain energy through extracellular electron transport to natural minerals or synthetic electrodes that serve as electron acceptors for respiration *outside* the cells. In addition to the fundamental implications for physiology and microbial communication, a physics-based understanding of this extracellular respiration will impact the transmission of signals at hybrid living/synthetic interfaces.

But how can a bacterium transport electrons to an external surface? In contrast to solid-state systems (e.g. metals and semiconductors), where the charge transport physics is well understood, comparatively little is known about the physics of biological charge transport over cellular length scales. In this talk I will describe how bacteria organize redox sites on outer cell membranes, and along quasi-one-dimensional filaments known as bacterial nanowires, to facilitate long-range charge transport. The approaches taken include microfluidic fluorescence assays, gene expression profiling, single-cell respiration measurements, scanning tunneling microscopy of redox molecules, and nanofabrication-enabled measurements of transport along individual bacterial nanowires produced by the bacterium *Shewanella oneidensis* MR-1. Based on these measurements, we propose that extracellular respiration is facilitated by an incoherent multistep charge hopping mechanism along redox chains. Finally, I will describe recent *in vivo* measurements of bacterial nanowire composition, extension, and redox activity. These measurements reveal surprising conclusions about the biomolecular makeup and function of bacterial nanowires.

Enhancement of energy recovery and pollutant degradation using a novel biocathode coupled photoelectrochemical cell (BIO-PEC)

Yujie Feng¹, Yue Du^{1,2}

A novel biocathode coupled PEC (BIO-PEC) was installed, integrating the advantages of photocatalytic anode and biocathode. Electrochemical anodized TiO₂ nanotube arrays fabricated on Ti substrate were used as Bio-PEC anodes. Biocathodes were enriched using nitrifying sludge and operated at various NH₄Cl and NaHCO₃ concentrations in both open and closed external circuit conditions. The system performance, including electrochemical impedance spectroscopy (EIS), MO degradation et al, showed that the biocathode had comparable catalytic activity with Pt/C cathode. The findings in this paper indicated that the biocathode was more dependent on the nutrients, such as nitrogen and inorganic carbon. Based on the variation of nitrification and cathodic oxygen reduction activity, it was shown that oxygen reduction process, to some extent, relied on the nitrification activity of biocathode.

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Scaling up biocathode microbial fuel cells

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Biocathode MFCs have the important advantages of low cost, good stability and multiple functions for wastewater treatment. Granular carbon packed bed biocathode MFCs have been investigated in our laboratory. Scaling up is a challenge to achieve practical application of MFCs. Selecting less-expensive electrode materials and current collector, optimizing anode and cathode thicknesses, and designing scalable modular reactors are key factors which should be taken into account in scaling up of MFCs. Compared with conventional electrode materials graphite and carbon felt, less-expensive activated carbon and activated semicoke were found to have higher power generation performance, lower cost, and higher operation stability, especially to low-strength wastewater. Multilayer metal mesh current collector could effectively transport electrons from electrode to external circuit, and the power generation performance was accordingly improved. The thicknesses of anode and cathode packed with granular electrodes were optimized. A scaled up pilot system stacked with three pairs of anode and cathode was fabricated. Different water flow and circuit connections were investigated to optimize the system performance. Results demonstrate that this stacked configuration of biocathode MFCs can be easily scaled up.

Hydrogenase-independent uptake and metabolism of electrons by the archaeon *Methanococcus maripaludis* Alfred M. Spormann¹, Svenja T. Lohner¹, Jörg S. Deutzmann¹, Bruce Logan², John Leigh³

Direct, shuttle-free uptake of extracellular, cathode-derived electrons has been postulated as a novel mechanism of electron metabolism in some prokaryotes that may be also involved in syntrophic electron transport between two microorganisms. Experimental proof for direct uptake of cathodic electrons has been mostly indirect and has been based on the absence of detectable concentrations of molecular hydrogen. However, hydrogen can be formed as transient intermediate abiotically at low cathodic potentials (<-414 mV) under conditions of electromethanogenesis. Here we provide genetic evidence for hydrogen-independent uptake of extracellular electrons. Methane formation from cathodic electrons was observed in a wild type strain of the methanogenic archaeon *Methanococcus maripaludis* as well as in a hydrogenase-deletion mutant lacking all catabolic hydrogenases, indicating the presence of a hydrogenase-independent mechanism of electron catabolism. In addition, we discovered a new route for hydrogen or formate production from cathodic electrons: Upon chemical inhibition of methanogenesis with 2-bromo-ethane sulfonate, hydrogen or formate accumulated in the bioelectrochemical cells instead of methane. These results have implications for our understanding on the diversity of microbial electron uptake and metabolism.

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ORAL PRESENTATION ABSTRACTS

Electrochemical mechanism of accelerated anodic respiration by *Shewanella oneidensis* MR-1 chemically modified with a membrane-intercalating conjugated oligoelectrolyte

<u>Nathan D. Kirchhofer</u>¹, Xiaofen Chen², James J. Sumner³, Enrico Marsili⁴, Frederick W. Dahlquist², Guillermo C. Bazan^{1,2}

Shewanella oneidensis MR-1 is a well-characterized model exoelectrogenic bacterium. Appropriately poised graphite electrodes serve as electrochemical proxies for the native respiratory substrates of *S. oneidensis*, and this allows for excellent electrochemical characterization in 3-electrode type reactors. It is now well understood that *S. oneidensis* utilizes a combination of direct electron transfer (DET) through the metal reduction (Mtr) pathway and flavin-based mediated electron transfer (MET) to achieve extracellular electron transport (EET). More recently, a growing body of literature indicates that chemical modification of microorganisms with micromolar concentrations of membrane-intercalating conjugated oligoelectrolytes (COEs) accelerates EET to an electrode acceptor. The universal observation of such EET acceleration would seem to be analogous to the natural EET observed in *S. oneidensis*, but up to this point, a straightforward mechanistic picture of COE-accelerated EET has remained elusive. Because *S. oneidensis* MR-1 undergoes both DET and MET, and the two processes are electrochemically distinguishable, this system serves as an excellent model organism for exploring exactly this COE-EET mechanism. This presentation will outline the 3-electrode amperometry and voltammetry experiments, as well as electron microscopy, that suggest that COEs accelerate EET from *S. oneidensis* by activating a subdued DET pathway without significant cytotoxicity. These results may have strong implications for understanding the effect of COEs on other species in bioelectrochemical systems.

Effects of additional electron acceptors in bio-anodic processes: oxygen and sulfate

Shiue-Lin Li^{1,2}, Kenneth H. Nealson¹

The availability of a terminal electron acceptor allows electron flow and sustains life of respiratory microorganisms. In an anodic process, the interactions between electron acceptors (i.e., electrode and the others) will affect the charge recovery and the current generation. To study complicated environments such as a seafloor, it is unlikely to remove all the alternative electron acceptors (e.g., sulfate, nitrate, and oxygen) when carrying out electrochemical analyses. Thus, investigating the effect of any additional electron acceptors should be the first task prior to field studies. Here we take two of our studying results for examples. A stronger extracellular electron transfer induced by oxygen on a *Shewanella* anode was elucidated. According to the results, a larger equivalent of nicotinamide adenine dinucleotide production was found in the presence of oxygen. It leads to a larger current generation but depresses the Coulomb efficiency. In addition to oxygen, sulfate is another alternative electron acceptor which can be utilized by sulfate reducing bacteria (SRB). Hydrogen sulfide generated via sulfate reduction could be reoxidized at the anode, forming element sulfur deposited. The use of cyclic voltammetry revealed that the sulfide started to be oxidized from 200 mV vs. Ag/AgCl, while the results of differential pulse voltammetry indicated indirectly that non-ionized sulfide is relatively difficult to be oxidized by the electrode. According to the electronic-microscopic image of SRB growing on graphite fibers poised at a potential of 400 mV (vs. Ag/AgCl), nanowire-like appendages could be found, which cannot be seen under the condition of

different potentials, we have concluded that many different organisms are involved, and we proposed that the mechanism(s) utilized will be revealed through transcriptional analyses of these enrichments done under different surface poised potentials.

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 \square 100 mV (vs. Ag

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Metabolic network in electrogenic biofilms identified by metatranscriptomic approach

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Microbial respiration via extracellular electron transfer (EET) is a ubiquitous reaction that occurs throughout anoxic environments and is a driving force behind global biogeochemical cycling of metals. Here we identify specific EETactive microbes and genes in a diverse electrogenic biofilm using an innovative stimulus-induced metatranscriptomic approach to analyze the dynamic community-wide gene expression response to changing EET rates. The anodic electrogenic community is established in microbial fuel cell fed with primary clarifier effluent in municipal wastewater plant. We applied two EET stimuli, set potential to +100 mV vs SHE for higher current generation and open circuit for zero current generation. We find that the most significant gene expression responses to applied EET stimuli occur in only two microbial groups, Desulfobulbaceae and Desulfuromonadales, which indicates that those microbes are EET-active and highly related to electrode conditions. On the other hand, highly gene-expressed microbes, methanogenic Methanosarcinaceae and sulfate-reducing Desulfobacteraceae are not responsive to the stimuli. Metagenomic analyses reveal high coverage twelve draft genomes of these abundant and active microbes, while metatranscriptomic analyses reveal that their metabolic network and the switch of metabolic pathways between the competitive microbes. Our metatranscriptomic results also show known and unknown genes that are highly responsive to EET stimuli and associated with our identified draft genomes. This new approach yields a comprehensive image of functional microbes and genes related to EET activity in a diverse community, representing the next step toward unraveling complex microbial roles and the metabolic network within a community and how microbes adapt to specific environmental stimuli.

Conversion of lignin to electric current with a defined co-culture of *Rhodopseudomonas palustris* and *Geobacter sulfurreducens*

<u>Devin F. R. Doud</u>¹, Largus T. Angenent¹

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Lignin, which is the second most abundant biopolymer on earth, is a complex and recalcitrant molecule that is currently produced as a waste by many industries. One of the primary components of lignin is p-coumaric acid, which can be degraded by the purple non-sulfur bacteria $Rhodopseudomonas\ palustris$ under anaerobic conditions. This catabolism has been well characterized and proceeds though a non- β -oxidation pathway. While metabolizing p-coumaraic acid, the NifA* mutant strain of R. palustris secretes both H_2 and acetate as metabolites. This mutant strain constitutively expresses its wild-type nitrogenase, producing H_2 to help maintain its intracellular redox balance. H_2 and acetate are substrates for the exoelectrogen $Geobacter\ sulfurreducens$ to produce current. In addition, the active scavenging of H_2 from R. palustris by G. sulfurreducens has the potential to exhibit synergy by enhancing rates of p-coumaric acid degradation in this system. Using this defined co-culture, R. palustris and G. sulfurreducens were grown at a poised electrode of a two chamber, 3-electrode bioelectrochemical system with R. palustris serving as a biocatalyst to convert p-coumaric acid to H_2 and acetate for consumption and current production by G. sulfurreducens.

Produced water treatment capability of lab and pilot scale microbial capacitive desalination systems

<u>Casey Forrestal</u>, Alexander Haeger, Louis Dankovich, and Zhiyong Jason Ren*

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Currently all across the United State unconventional natural gas development has led to the generation of a hydrocarbon and saline rich wastewater called produce water. Produced water is difficult to treat using conventional methods because it contains salts and hydrocarbons. Using a recently developed method called microbial capacitive desalination (MCD) both the organic carbon and total dissolved solids (TDS) can be removed

from the produced water. Here we present the results of a lab scale reactor, which was able to remove total dissolved solids (TDS) at the rate of 2,760 mg TDS/L/hr and chemical oxygen demand (COD) at a combined rate of 170 mg COD/L/hr. Moreover, we recently developed a first pilot scale system consists of multiple MCDs, a peristaltic pump, an energy harvesting system, 7 sensors, data collection and an automation control system. The pilot system is operated at flow rate of 0.6L/min (228 gallons/day), and is capable of removing 0.3 mg COD/L/min (473 mg COD/L/Day) from the anode chamber and 0.5 mg COD/L/min (724 mg COD/L/day) from the desalination chamber. From the desalination chamber 66% of the TDS was removed at a 50% water recovery rate. The highest power density achieved from a single MCD was 89 W/m³ with a coulombic efficiency of 11.4%.

Improving coulombic recovery in fermentable substrate fed MECs by shifting the products of fermentation Joseph F. Miceli III^{1,2}, César I. Torres^{1,3}, Rosa Krajmalnik-Brown^{1,4}

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Applying MXCs to complex organic materials often requires fermentation by mixed cultures in order to convert the initial substrates into acetate. Mixed culture MXCs fed with fermentable substrates typically lose 20-50% of the incoming electrons to the production of methane, using hydrogen as an intermediate. Hydrogen production is favorable during fermentation as long as its concentration remains low, a state typically maintained by hydrogenotrophic methanogens. Due to the thermodynamics of the reactions involved, when hydrogen concentrations rise, more fatty acids and alcohols are produced instead of hydrogen. Therefore, we hypothesized that feeding high concentrations of fermentable substrates in batch mode will improve the efficiency of MXCs. Using batch fermentation cultures fed with varying concentrations of five different electron donors, we first determined concentrations at which hydrogen production is inhibited. We then established two-chamber microbial electrolysis cells (MECs) with potentiostatically controlled anodes (-0.3V vs Ag/AgCl), inoculated with anaerobic digested sludge, and fed either ethanol, lactate, or glucose at concentrations of 300 me- eq./L, in duplicate. The MECs were monitored for fermentation products and current. The fraction of electrons converted to current from the initial electron donor was 73-74% for glucose, 75-77% for ethanol, and 78-81% for lactate. Losses to methane ranged from 1 to 7% across the various reactors, these losses are significantly lower than previous reports. Coulombic recovery improved ~50% over conventional feeding regimes using fermentable substrates. This work demonstrates how the manipulation of substrate loading can lead to shifts in the metabolic intermediates produced during fermentation. We demonstrated how instead of producing hydrogen (and losing electrons to methanogenesis) acetate, propionate, and butyrate were generated leading to greater efficiencies in the MECs. This outcome is important and can be applied to MXCs generally as well as other fermentation based systems such as hydrogen or methane production from biomass.

Characterization of the simultaneous fermentation and anode respiration capabilities of the thermophilic Thermoanaerobacter pseudethanolicus in a Microbial Electrolysis Cell (MEC)

Qaiser F Khan³, Bradley G Lusk¹, <u>Prathap Parameswaran</u>¹, César I Torres^{1,2}

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Thermoanaerobacter pseudethanolicus (ATCC No: 33223) is a thermophilic gram-positive, spore forming anaerobic bacterium from the genus *Thermoanaerobacter* with capabilities to ferment carbohydrates such as glucose, xylose, cellobiose etc., while coupling it with Fe(III) reduction. We evaluated the anode respiration potential of T. pseudethanolicus using an H-type microbial electrolysis cell (MEC) operated at 60 $^{\circ}$ C at a poised anode potential of -0.06 V (vs) SHE. The four substrates evaluated as electron donors were xylose, cellobiose, glucose, and acetate which led to rapid fermentation (except for acetate) to produce mixed volatile acids and alcohols such as acetate, lactate, iso-butyrate, and ethanol. This was accompanied by consumption of the fermentation products by T. pseudethanolicus on the biofilm anode, with maximum current densities in the following order: glucose (5 A/m²) <

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cellobiose (4.8 A/m²) < xylose (4 A/m²) < acetate (0.03 A/m²). Low Scan Cyclic Voltammetry (LSCV) of fully grown biofilms revealed sigmoidal response indicative of Nernst-Monod behavior, with a mid-point potential of -0.18 V (vs) SHE for a xylose fed biofilm anode. While confocal laser scanning microscopy confirmed the presence of a thick biofilm ($^{\sim}$ 150 μ m), scanning electron microscopy revealed physiological features in the biofilm that were indicative of a complex extracellular matrix. This is one of the earliest documentations of a microbe with the capability to perform both fermentation and subsequent anode respiration on the fermentation products at elevated temperatures, which makes *T.pseudethanolicus* a candidate for renewable energy capture.

Cathodes of microbial electrolysis cells (MEC) support preferential growth of methanogens

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In two-chamber methanogenic MECs, carbon dioxide (CO_2) is reduced to methane (CH_4) by microorganisms growing on cathodes that are able to use electrical current or hydrogen gas. Here we tested different cathode materials (metals and minerals on carbon black, graphite and carbon brushes) to investigate their impact on methane production and microbial community structure. The cathodes were inoculated with anaerobic sludge from a wastewater treatment facility. We discovered that after 5 batch cycles, graphite electrodes had the same performance as platinum on carbon black when poised at -550 mV. At -600 mV, carbon brush reactors showed the highest performance after platinum. A significant shift of the microbial community compared with the inoculum occurred during incubation. While methanogenic *Archaea* made up less than 4% of the microbial population in the inoculum, their proportion increased to a median of 31% during incubation. In the catholyte, the archaeal population increased to 42%. However, the total number of microbial cells was two times higher (median) on the cathodes than in the catholyte, with extreme outliers of 13 times higher for platinum and 12 times higher for iron sulfide compared with the medium. This indicates that microorganisms preferentially adhered to carbon black cathodes doped with platinum and iron sulfide. These two materials also reached their full methane production performance during the first cycle (Pt: 250 nmol ml $^{-1}$ d $^{-1}$ and FeS: 40 nmol ml $^{-1}$ d $^{-1}$) and are like carbon brushes (100 nmol ml $^{-1}$ d $^{-1}$) well suited for cathodic methane generation.

Developing microbial electrochemical systems for electricity and hydrogen production in biorefinery Abhijeet P. Borole^{1,2}, Daniel J. Schell³

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Biomass conversion to biofuels can be achieved via biochemical and thermochemical processes at a process efficiency of 45-60%. A significant portion of the biomass is converted to byproducts and other compounds, some of which are inhibitory to the biofuel production processes. Water recycle is an important consideration for improving resource utilization in the biorefinery. The fermentation inhibitors generated during biomass pretreatment and conversion accumulate in the process stream if the water is recycled without treatment. Presence of sugar- and lignin- degradation products such as furfural, vanillic acid and 4-hydroxybenzaldehyde has been shown to drop fermentation yields precipitously. In this work, we demonstrate the potential for electricity and hydrogen production from these compounds using microbial fuel cells and electrolysis cells. Estimates for bioenergy production via such systems in full-scale biorefineries will also be presented. Production of hydrogen as an alternate product can have a profound impact on the biorefinery process efficiency and economics. Challenges to implement this in the biorefinery including scale up of these systems will be addressed.

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Systems biology approach to characterize multispecies biocathode biofilms

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Electric bacteria can exchange electrons through extracellular electron transfer (EET) with electrodes as insoluble electron donors or acceptors. The ability of some bacteria to oxidize an electrode may be conferred by a natural ability to oxidize insoluble electron donors in the environment. Such electron transfer processes are desirable for a number of applications, including improving microbially-mediated O₂ reduction in MFCs and bioelectrosynthesis. Biocathodes in the marine environment must be robust in order to tolerate high salinity, changing atmospheric conditions (including O₂ and CO₂), and defense from predation. Here we apply cultivation independent techniques to allow us to explore the natural environment for new biocathode catalysts and EET components. We present results from a tri-omics, systems level approach to study an autotrophic multispecies marine biofilm consuming electricity from an electrode (+310 mV SHE) and fixing CO₂ from seawater. Metagenomic analyses (Illumina HiSeq 1000) by sequence composition and sequence similarity based methods are consistent with a community divided between Alpha- and Gammaproteobacteria. Metatranscriptomic (Illumina MiSeq) and metaproteomic analyses (LC-MS/MS) indicate an unknown member of the order Chromatiales and a Marinobacter sp. are most abundant and relevant for electron transfer and CO2 fixation, accounting for over 70% of observed proteins in these functional categories and correlating with gene expression patterns. Proteins for key components of a putative microbial iron oxidation pathway, including Cyc2/Cyt572 and MopB, were identified in Marinobacter and Chromatiales and mutagenesis and heterologous expression studies are underway. Electrochemically active biofilm isolates included Marinobacter, Alcanivorax, and Labrenzia spp., however, isolates could not sustain electrical activity without introducing organic carbon and vitamins indicating biofilm community is required. These results demonstrate potential use of a naturally occurring, self-assembling and self-regenerating electrode biofilm as a robust, multispecies chassis for synthetic biology applications in the marine environment where existing, pure culture chassis organisms may not be applied.

Continuous production of an oily stream of medium-chain carboxylic acids with a microbial electrochemical technology

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Using sustainable feedstocks for fuel and chemical production is increasingly viewed as an economically viable and environmentally responsible approach to minimize our consumption of fossil fuels. Fermentations with reactor microbiomes can produce medium-chain carboxylic acids by elongating short-chain carboxylic acids from organic wastes through the addition of ethanol as a source of reducing equivalents, energy, and carbon. However, the medium-chain carboxylic acids must be separated from the effluent of the bioreactor, which adds operating costs. Our previous work showed that a liquid/liquid extraction (pertraction) system removed *n*-caproate from the fermentation broth by accumulating it into a basic solution. Here, we developed a microbial electrochemical technology (MET) by integrating an abiotic membrane electrolysis cell (similar to Andersen, S; Hennebel, T; Gildemyn, S; Coma, M; Desloover, J; Berton, J; Tsukamoto, J; Stevens, C; Rabaey, K, Electrolytic membrane extraction enables fine chemical production from biorefinery side streams; Submitted) with the fermentor and pertraction system. This MET converted beer from the ethanol industry with 15% ethanol into an oily substance that contained >92% carboxylic acids (*n*-butyric acid, *n*-caproic acid, and *n*-caprylic acid). The basic pertraction solution was recirculated through the cathode chamber of the membrane electrolysis cell, resulting in the selective

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transfer of charged carboxylates through the anion exchange membrane from the basic catholyte to the acidic anolyte. Due to the accumulation of n-caproic acid (low pH) at concentrations that exceeded the maximum solubility, phase separation was achieved in the anolyte, which is the novelty of this work that will be presented here. We achieved a coulumbic efficiency of 60% and an n-caproate membrane flux of 620 g m⁻² d⁻¹ at a current of 10 A m⁻². This system also has the added benefit of considerably lowering the pH-control costs by recycling OH into the pertraction and fermentation system.

Molecular characterization of the interactions that mediate electron transfer across the microbe-electrode interface in *Shewanella oneidensis* MR-1

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Bioelectrochemical technologies (BETs) represent a new industrial paradigm for novel applications in wastewater treatment, sustainable energy production and even synthesis of high added value compounds. In applications of these technologies, microorganisms support their growth by oxidizing organic compounds and an electrode serves as the sole electron acceptor where electricity can be harvested. Shewanella oneidensis MR-1 (SOMR1) is one of the best studied organisms that are capable to power up BETs. The electron transfer pathway of SOMR1 to inorganic minerals and metals is sustained by several c-type multiheme cytochromes that shuttle electrons from cytoplasm and inner membrane oxidizing enzymes toward the outside of the cell. Among these proteins the outermembrane decaheme cytochromes MtrF, MtrC and OmcA were shown to be responsible for both direct and indirect electron transfer at the microbe-mineral interface. Riboflavin and FMN were identified as soluble electron shuttles that mediate extracellular electron transfer in SOMR1, facilitating electron transfer to both metals and electrodes, by interacting with the outer-membrane cytochromes. Nuclear Magnetic Resonance spectroscopy (NMR) was used to obtain molecular insights on the interaction between these outer-membrane cytochromes and flavins, allowing, for the first time, the characterization of interaction between the terminal oxidoreductases of SOMR1 and flavins. These results showed that these proteins, although homologous and modular, have distinct modes of interaction with extracellular flavins, shedding light into functional specificity of the outer membrane oxidoreductases responsible for extracellular electron transfer. Being one of the primary mechanism of extracellular electron transfer employed by SOMR1, understanding the molecular details of flavin electron shuttling, will provide support for the rational improvement of electrode materials and design for application in BETs.

Metabolic characterization of anode-respiring Escherichia coli

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Combining electrode communication with the synthetic biology toolkit available for *Escherichia coli* opens the door to many new bioelectrochemical applications, particularly in biosensing, hybrid robotics, and electrosynthesis. We previously developed *E. coli* strains capable of generating electric current at an anode by incorporating the membrane spanning electron conduit (Mtr pathway) of *Shewanella oneidensis* MR-1. However, it was unclear if the engineered *E. coli* was respiring anaerobically using the anode or if it was fortuitously donating electrons to the electrode from other catabolic pathways. Because this question is critical to developing new technologies, we must determine the metabolic pathway driving the electron transfer and the efficiency of current production. We grew the engineered *E. coli* with and without poised electrodes and measured growth, coulombic efficiency, and the production of key metabolites and energy carriers. Our results suggest that our strains are capable of electrode-supported growth at a low coulombic efficiency. However, the intracellular rate of electron transfer into the Mtr pathway limits the metabolic rate, and thus current production, in these strains. This limitation may perturb redox balance in the cell and encourage the production of reduced metabolic byproducts such as ethanol and acetate instead of CO₂. This work will yield insights into the metabolism of anode-respiring organisms and methods for shunting electrons toward information transfer and biosynthesis of products.

Combined bioelectrochemical-electrical model of a microbial fuel cell

<u>Didac Recio Garrido</u>¹, Michel Perrier¹ and Boris Tartakovsky²

Dynamic mathematical models represent a useful tool in portraying unsteady-state behavior and understanding fundamental properties of microbial fuel cells (MFCs). Recently developed models are able to adequately describe long-term behavior of MFCs operated with a fixed external resistance. These models, however, do not account for the double layer capacitance effect and complex non-linear dynamics observed in most recent experiments, in particular tests involving pulse-width modulated connection of the external resistance (electrical load). To further extend our understanding of MFC dynamics, a combined bioelectrochemical-electrical model (CBE model) obtained by adding fundamental equations based on mass and electron balances with equations describing an equivalent electrical circuit has been developed.

The CBE model of an MFC considers two microbial populations (electricigenic and methanogenic) with kinetics governed by double Monod terms. Anodic biofilm formation is accounted for by limiting the maximum attainable concentration of the microorganisms. Direct contact, nanowires or a mediator-based electron transfer mechanism from the carbon source (acetate) are assumed and the electron transfer by the electricigenic bacteria is considered to involve the oxidized and reduced forms of an intracellular mediator (e.g. NADH/NAD[†]). On the other hand, a non-limiting cathode reaction rate is assumed. Finally, the CBE model describes the double layer capacitance, which is assumed to be related to the accumulation of biomass at the electrode and is described by a corresponding capacitor dynamic equation incorporated into the electrochemical balance. Consequently, CBE model is capable of describing both fast (milliseconds) and slow (hours and days) dynamic behavior observed in MFCs.

Harvesting microbial fuel cell ionic currents for enhanced energy generation through capacitive mixing Marta C. Hatzell'¹, Roland D. Cusick² & Bruce E. Logan³

The recent combination of salinity gradient energy technologies (e.g. reverse electrodialysis (RED)) with microbial fuel cells was shown to significantly boost the power from bioelectrochemical systems (BESs) by nearly 5 times. Yet RED suffers from high cost and material lifetime issues with the ion exchange membranes. Capacitive mixing (CapMix) is an alternative technology for capturing salinity gradient energy which harvests energy through the controlled ion transfer to and from capacitive electrodes. CapMix has suffered from low power densities, but is still promising due to the low cost and long materials lifetime. Experiments were conducted to investigate the synergy between BESs and CapMix. The combined system was shown to increase the energy harvested from the CapMix process with synthetic sea and river solutions by 60 times from 0.04±0.003 mJ cycle⁻¹ to 2.64±0.240 mJ cycle⁻¹ with a constant BES current of 4 mA. Power densities achieved through the capacitive mixing cell increased by 50 times from ~10 mW m⁻² (without BES) to ~500 mW m⁻²(with BES). These results represent a significant increase in both the energy extracted and power densities achieved through CapMix technologies.

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Effect of physical and geometrical properties of carbon nanotube anodes on microbial fuel cells

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Maximizing contact and minimizing electron loss between electrochemically active bacteria (EAB) and anodes of microbial fuel cells (MFCs) are one of the routes to improve MFC power performances. Various nanomaterials such as gold nanoparticles, graphenes, and carbon nanotubes (CNTs) have been used in recent years to improve the physical and electrochemical interactions at the EAB-anode interface to improve MFC power output. However, how the physical and geometrical properties of these nanomaterials affect power output have not been fully studied. Here we attempt to investigate how different lengths, packing densities, and surface conditions of CNTs used as anodes affect MFC power output. The electrodes consist of multi-wall carbon nanotubes (MWCNTs) directly grown from the wires of stainless steel (SS) meshes through a chemical vapor deposition (CVD) method, providing extremely large three-dimensional surfaces while ensuring minimal ohmic loss between CNTs and SS meshes. Electrodes with different CNT lengths (~8 μm vs. ~19 μm), packing densities (loosely packed vs. aligned and densely packed), and surface conditions (amorphous carbon layer vs. pure CNT) were successfully synthesized by changing the CVD processing conditions. The power density from the long CNTs (~19 μm) was 450 mW/m², which was 28% higher than that from short CNTs (~8 μm) (350 mW/m²). Since the active surface area of long CNTs is 82% larger than that of short CNTs, it is thought that a larger number of microbes can interact with the electrodes having longer CNTs. Another key aspect in facilitating EAB-electrode contacts is the accessibility of microbes to the CNT surfaces. When highly aligned and densely packed CNTs were used, the MFC power output was 57% lower compared to that of the loosely packed CNTs. This is probably due to the highly aligned and densely packed CNTs preventing microbes from intercalating into the CNT forest, as shown through scanning electron micrographs. Lastly, an amorphous carbon layer coating on CNTs, which can be commonly found if CVD synthesis conditions are not optimized, resulted in 50% decrease in power. Since graphitic layer of CNTs often makes relatively strong bonding between organic molecules due to the dangling bond and π - π interactions, the amorphous carbon layer covering the graphitic layer would result in a weaker bonding and thereby a larger ohmic loss. These systematic studies clearly show the importance of characterizing and optimizing nanomaterial electrodes, including CNT-based ones, to maximize the effect of nanomaterial anodes for improved MFC performances.

Stable and high voltage and power output of microbial fuel cells internally connected in series

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The voltage output of a single MFC is normally less than 0.8 V, often less than 0.4 V at maximum power output, which greatly limits the application of MFCs. Serially connecting several MFCs together using a conductor or current collector, or using a DC/DC converter to boost voltage can increase the voltage output. These methods, however, not only lead to increased complexity and/or cost, but can lead to problems like voltage reversal and/or voltage cross-over, and reduced overall efficiency. Here we present a new configuration, microbial fuel cells internally connected in serial (iCiS-MFC), in which the anode and the adjacent cathode share the same base material. An iCiS-MFC equivalent to 4 regular MFCs produced a high voltage output of over 2 V. The maximum power densities of the iCiS-MFC reach over 3 W/m² in the lab with synthetic media and 1 W/m² in situ with real brewing wastewater. The new configuration shows great promise towards practical application of MFCs as it enhances the stability and performance of MFCs while reducing their complexity and cost.

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High performance monolithic power management IC chip system with dynamic maximum power extraction algorithm for microbial fuel cells

<u>Celal Erbay</u>¹, Salvador Carreon-Bautista¹, Edgar Sanchez-Sinencio¹, and Arum Han^{1,2}

Low voltage and low power production of MFCs make it difficult for them to be directly used as power sources in most practical applications. Power management systems (PMSs) are designed to overcome such problems by processing MFC power and voltage to a more readily usable level by up-converting the voltage and storing the generated power to external energy storage devices such as supercapacitors. We present the development of a low-power monolithic PMS integrated circuit (IC) chip system with an embedded maximum power extraction algorithm (MPEA). The system allows continuously detecting the maximum power point (MPP) of an MFC to maximize power extraction without using multiple external resistors to find MPP, which is inherently slow and power consuming. The PMS presented here delivers a fixed up-converted voltage from low and fluctuating voltages produced by an MFC at MPP to a supercapacitor, from which electronic applications such as wireless sensors for autonomous operation can be directly powered. Along with the MPEA system, the PMS is composed of a DC-DC boost converter operating in discontinuous conduction mode (DCM) to maximize efficiency without any external power source to operate the PMS. A supercapacitor (100 mF) was charged to 2.5 V using the presented PMS controlling an MFC that produced 328 µW of power (240 ml two-chamber MFC using wastewater) at MPP, and successfully operated a wireless temperature sensor (consuming 87.5 mW during operation). After the PMScontrolled MFC powered the sensor to send the temperature reading wirelessly to a base station, the voltage dropped to 2 V, and it took 12.5 minutes to recharge the supercapacitor to 2.5 V, thus providing wireless temperature reading every 12.5 minutes. The overall system efficiency, defined as the ratio between the output energy stored into the supercapacitor of the PMS and the input energy from the MFC, was 22.2%. The monolithic IC-chip based PMS presented here is expected to provide a low-power consuming highly efficient power management solution for MFCs.

High performance of spirally-wound microbial fuel cell design and its analysis using interdisciplinary methods Alexander Haeger, Casey Forrestal, Zhiyong Jason Ren*

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Bioelectrochemical systems (BES) have been studied at great length and there is an expansive knowledge base of their functionalities and basic performance benchmarks. Despite the attractiveness of BES, scalability is often the elephant in the room when it comes to increasing their throughput. Volumetric power along with the electrode surface area to volume ratio have provided a great base for comparing reactor designs in terms of overall performance and scalability, however as reactors are becoming increasingly complex more in depth analysis is needed to sufficiently assess them. An improved catalyst-free spirally-wound microbial fuel cell (swMFC) design has been developed, benchmarked, and analyzed in this study according to a broad range of engineering principles. Road blocks to the manufacturability of swMFCs were overcome in this study with the development of a new manufacturing process as well as a thorough sealing scheme. Further, the internal hydraulics of the new swMFC were analyzed using tracer studies to elucidate anode effective volume, or the actual volume of the anode that sees anolyte flow, which is thought to correlate to power production. Finally, standard electrochemistry tests were conducted to determine internal resistance, volumetric power, and Coulombic efficiency at three hydraulic retention times. Combined, the suite of analysis conducted on the improved and catalyst-free spirally-wound design has shed a broader light on performance and scalability than ever before. Two swMFC with equal total anode volumes were constructed for this study and proven to act as nearly plug-flow reactors according to tracer study results. It was also shown that volumetric power increases to over 50 W/m³ with increasing anode electrode surface area, despite corresponding decreases in effective anode volume. This study presents an improved spirally-wound design along with a suite of analysis to prove improved performance over previous designs and highlight room for design improvements.

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Challenges in controlling methanogens in microbial electrolysis cells

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Microbial electrolysis cells (MECs) can be used for sustainable wastewater treatment as organic substrates are oxidized at the MEC anode and the cathode electrolyzes water to produce H₂ gas. Produced H₂ gas in MECs is often converted into methane by H₂-oxidizing methanogens. Also, H₂-oxidizing methanogens grow rapidly compared to other anaerobic microorganisms. As a result, the H2 yield and purity in MECs can be seriously diminished. In this study, we focused on controlling methanogenesis using an electrolytic oxygen generation system. When a lab-scale MEC was constructed, the MEC cathode was prepared with two pieces of stainless mesh. Both pieces were used to induce the H₂ evolution reaction while the bioanode oxidizes organic substrates during Normal operation (Eap = 0.8 V). During Oxygen operation (Eap = 3.5 V) with the bioanode disconnected from the electric circuit, one piece of the stainless steel mesh was remained cathodic for H₂ evolution but the other piece was used as an abiotic anode for O₂ production. This intermittent exposure of pure oxygen in MECs successfully inhibited methane production over a month. Experimental results showed that effective methane control was depended on the frequency of Oxygen operation, requiring an oxygen exposure at least every 12 hours. We also performed a model study to simulate the rate of methanogenic growth based on the Monod equation and previously reported kinetic constants. Model results indicated that H₂-oxidizing methanogens in MECs can multiply their population by more than 5 orders of magnitude over three days. Because of this rapid growth of H2-oxdizing methanogens, the frequent oxygen exposure was essential for the control of methane contamination in gas recovered from MECs. Other difficulties we experienced in controlling methanogens will also be shared in the presentation.

Nano/micro/macro bioelectronics platform utilizing for scale-up of bio-electrochemical cell

Bongkyu Kim¹, Junyeong An^{1,2}, Daehee Kim^{1,3}, and In Seop Chang¹

To follow trend of bio-electrochemical system, a microbial fuel cell (MFC) have improved to build-up strategy which allows increase practicality of scaling-up system to application at actual filed. To build-up the strategy, scaling-up MFC has to consider about two aspects: 1) how to increase system scale, and 2) how to maintain performance efficient compared with lab scale. To achieve the strategy, in this study several types of MFC construction have been suggested and developed. Modulation concept was suggested to increase the practicality of lager operation system construction which consists of modular and stack system that challenge for the original formation of MFC with performance maintenance. In addition, it was studied that series/parallel connection of MFCs for developing suitable operation method to impregnate into modulation concept for enhancement of power output. Finally according to the preceding concepts, the new concept of MFC construction was designed with unique BEA structural called a bipolar plate electrode assembly. The suggested new concept was relatively simple and cost effective because it uses an air-exposed cathode with no membrane, enabling the compact MFC system to be modularized in a series circuit without wiring system. Besides, it was researched about several catalysts to develop suitable cathode for improving performance limitation generated at cathodic reaction in new concept. Hence, this newly novel structure of MFC could be successfully applied to actual filed with increased system size by modulation and enhanced performance by stack.

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Examination of spatial characteristics and gap-bridging ability of conductive mixed species biofilms

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Recent studies have suggested that some biofilms associated with microbial electrochemical systems exhibit conductive properties. While certain bacteria such as *Geobacter sulfurreducens* have been shown to produce conductive filaments conferring conductivity to axenic biofilms, mixed species biofilms have also exhibited conductivities similar to those pure cultures. In the current study, a mixed culture known to generate high power densities was examined after growth on gold-coated anodes with non-conductive gaps from 50 to 1000 μ m to evaluate the spatial characteristics of this conductivity. Biofilm conductivity was monitored by *in situ* 2-probe measurements, while biofilm thickness was characterized by confocal laser microscopy. Community analysis was also conducted to provide comprehensive information about bacterial composition. Though the microbial community was not dominated by *Geobacter spp.* (22% of total 16S reads), conductive biofilms were still produced and able to span non-conductive gaps 200 times longer (1000 μ m) than average cell length (5 μ m). This research sheds light on a unique characteristic of mixed species biofilms and will help guide future innovations of microbial electrochemical systems.

POSTER PRESENTATION ABSTRACTS

Energy generation from domestic wastewater using a custom made double chamber microbial fuel cell JA Adeniran¹, R Huberts², JJ De-Koker³, O Arotiba⁴, E Van-Zyl⁵ and S Du-Plessis⁶

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A locally designed dual purpose double chamber Microbial Fuel Cell (MFC) was used for energy generation and domestic wastewater treatment. The MFC was constructed by forming a sandwich of anode, proton exchange membrane and cathode (mesh) and thereafter glued to the anode chamber. The power produced by the reactor was determined by measuring the potential across the external resistor through the connected digital multimeter. Maximum power density of 213 ± 14 mW/m² and current density of 0.054 ± 0.002 mA/cm² were obtained using full strength domestic wastewater (3400 mg/L of COD), at an external resistance of 200Ω , resulting in a maximum COD removal of 89%. This indicates that the reactor was efficient in terms of wastewater treatment. The power and current densities dropped to 60 mW/m^2 and 0.03 mA/cm^2 respectively using wastewater of a lower COD concentration (300 mg/L), representing a decrease of 70% in power density. The coulombic efficiency range from 5% to 7% suggests that the design needs to be further optimized for increased performance. The use of full strength natural domestic wastewater reduces the cost of MFC experiments and with further improvement on the reactor design, the ultimate goal of using natural wastewater for energy generation and treatment of wastewater using MFC on a large scale is achievable in the future.

Effects of localized surface pH on the metabolic activity of attached bacteria

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Bacteria have acidic and basic functional groups at the cell surface and during the process of adhesion onto a solid surface, which also can have functional groups, the charge-regulation effect results in changes in the functional group speciation and local pH due to the electroneutrality requirement. The local pH can either rise or fall, depending on the specific functional groups and their site densities on the two surfaces. It has been observed that the metabolic activity of attached bacteria can differ from that of their planktonic counterparts, and we have developed a working theory relating bacterial activity to the changes in local pH that occur upon adhesion. Specifically, the local pH change during adhesion affects the proton gradient across the cell's cytoplasmic membrane. This proton gradient controls the concentration of the energy carrier adenosine triphosphate (ATP); thus, changes in local pH at the cell surface can affect cellular ATP levels and metabolic activity. The hypothesis predicts that a decrease in local pH should result in higher ATP levels, while an increase in pH should result in lower ATP levels.

Here, we will present numerical and experimental results demonstrating how surface acid/base functional groups can alter the local pH of attached bacteria and how this results in variations in cellular ATP concentration. Experimental results will be discussed that demonstrate both enhanced and inhibited metabolic activity. The long-term goal is to provide a means to interpret bacterial interactions with different surfaces and to provide a basis for the design of materials exhibiting desired bacterial activities.

Inhibitory effects on the cathodic bio-film of a denitrifying bio-cathode microbial fuel cell

A. Al-Mamun¹ and How Yong Ng²

Denitrifying bio-cathode Microbial Fuel Cell (MFC) is a complete Bio-electrochemical system (BES) where both the anodic oxidation and the cathodic reduction reactions are catalyzed by micro-organisms. In this study, we developed a three-chambered horizontal and up-flow channel MFC where the middle chamber acts as denitrifying bio-cathode and the two chambers at the side act as biological anode. Graphite granules act as electrodes in both anode and cathode chamber and nafion membranes were used as separator between the chambers for proton diffusion. The maximum volumetric power obtained was 14.63 W.m⁻³ NCC (Rext = 11.5 Ω) at a cathodic nitrate loading of 0.15 kg NO₃⁻N.m⁻³ NCC.d⁻¹, indicating the potential of using denitrifying bio-cathode MFC for energy production. The obtained maximum power was approximately 83% higher than that obtained by Clauwaert et al. (2007b). Complete denitrification from nitrate (NO₃⁻) to molecular nitrogen (N₂) is achieved by four reduction steps forming nitrite (NO₂⁻), nitric oxide (NO) and nitrous oxide (N₂O) as intermediates. The details of the biochemistry of biological denitrification processes are shown in Figure 1 with the redox potentials and enzymes of each step.

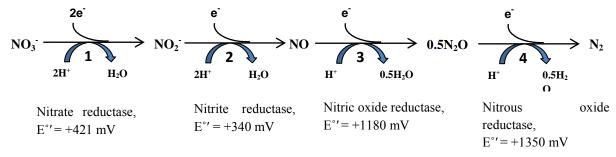


Figure 1: Biochemistry of biological denitrification with redox potential (E°′) (Kim and Gadd, 2007).

The inhibitory effect of the intermediate denitrifying product- nitrite (NO_2) was observed. The formation of nitrite is associated with the formation of Free Nitrous Acid (FNA) in the aqueous chemical system. The experimental analysis showed that the observed degree of inhibition correlated much more strongly with the FNA, rather than nitrite concentration, indicating FNA as the true inhibitor on the activity of denitrifying microbes. The results showed that both the current generation and the denitrification activity were decreased at a cathodic NLR of more than 0.175 kg NO_3 -N.m⁻³ NCC.d⁻¹. Approximately 45% of the current production and 20% of the total denitrification activity in this bio-cathode MFC was decreased at a FNA concentration of 0.0014±0.0001 mg HNO₂-N.L⁻¹ (equivalent to the nitrite concentration of 6.2±0.9 mg NO_2 -N.L⁻¹ at a pH of 7±0.1). These results show that the autotrophic denitrifying bacteria are more tolerant than the heterotrophic denitrifying bacteria (Zhou Y. et al., (2008).

Novel architecture for modulation of membrane-less microbial fuel cell with bipolar-plate electrode assembly (BEA)

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Microbial Fuel Cells (MFCs) are well known as a promising technology for wastewater treatment process, but they suffer from two main restrictions. 1) non-cost effective materials such as membrane and noble metal catalysts, and 2) difficulties for scaling-up. In order to overcome these issues, Membrane-less MFC with Bipolar-plate Electrode

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Assembly (BEA) has been developed in our study. Especially, structure of BEA is an important design for scaling up system, which is able to modularize stackable MFCs with series connection. The reason is that rigid-waterproof graphite plate as interface electrode of BEA can allow the anode on this plate in MFC unit to be stacked on top of cathode in another MFC unit, which is connected in series via BEA without additional connections by external wires. It also prevents disturbing activity of electrochemical active bacteria (EAB) on anode surface from diffusion of oxygen at outside. The maximum power of stack MFCs with series connection was about 2.5 times higher than the each MFC unit. However, one factor should be considered to increase maximum power of stack MFCs, which is a possibility of a formation of cathodic limitations in MFC unit. Because a part of the cathode of BEA is directly contacted with organic rich solution without using membrane, it becomes a critical cause that lower Oxygen Reduction Reaction (ORR) kinetics of cathode part. Following study is still ongoing to improve cathodic limitations of stack MFCs. In conclusion, this concept clearly suggests cost effective MFC for scaling-up because BEA without membrane enables the development of a compact MFC system to be modularized in a series connection without wiring.

The mechanism of rapid redox flexibility in a sediment-derived electrogenic biofilm community

<u>Tyler J. Arbour</u>¹, Kelly C. Wrighton¹, Sean W. A. Mullin¹, Cindy Castelle¹, Birgit Luef¹, Benjamin Gilbert², and Jill F. Banfield^{1,2}

As a response to changes in mineral redox potential, microbial communities may shift their membership, or individual organisms may adjust protein expression. Alternatively, the ability to respond may be conferred by the innate characteristics of certain electron transport chain components. Here, we used potentiostat-controlled microbial fuel cells (MFCs) to measure the timescale of response to imposed changes in redox conditions, thus placing constraints on the importance of these different mechanisms. In the experiments, a solid electrode acts as an electron-accepting mineral whose redox potential can be precisely controlled. We inoculated duplicate MFCs with a sediment/groundwater mixture from an aquifer at Rifle, Colorado, supplied acetate as an electron donor, and obtained stable, mixed-species biofilms with Geobacter species making up the largest component. We poised the anode at potentials spanning the range of natural Fe(III)-reduction, then performed cyclic voltammetry (CV) to characterize the overall biofilm redox signature. The apparent biofilm midpoint potential shifted directly with anode set potential when the latter was changed within the range from about -250 to -50 mV vs. SHE. Following a jump in set potential by 200 mV, the CV-midpoint shifted by ~100 mV over a timescale of ~30 minutes to a few hours, depending on the direction of the potential change. The extracellular electron transfer molecules, whose overall CV signature is very similar to those of purified multiheme cytochromes (MHCs), appear to span a broad redox range (~200 mV), supporting the hypothesis that MHCs confer substantial redox flexibility. This flexibility may be a principle reason for the abundance of MHCs expressed by microorganisms capable of electron transfer to minerals.

Changes in reaction pathways in electrically-assisted anaerobic digesters

Joseph Asztalos¹, Younggy Kim²

Biosolids destruction is slow in anaerobic digesters and thus digesters are operated at a long retention time (10-20 days). Acetate-oxidizing methanogenesis is the rate-limiting reaction requiring at least 10 days for effective conversion of acetate into methane. In contrast, other anaerobic reactions (hydrolysis, fermentation, beta oxidation, and H₂-oxidizing methanogenesis) are driven in ~6 days. Thus, anaerobic digestion can be achieved in 6 days if acetate-oxidizing methanogenesis is replaced by other rapid reactions. The main objective of this study was to investigate how the reaction pathways are affected in biosolids destruction when microbial electrolysis cell (MEC) technology is implemented in anaerobic digesters to replace acetate-oxidizing methanogenesis. At the MEC anode, exoelectrogenic bacteria oxidize short chain fatty acids (including acetate), replacing acetate-oxidizing

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methanogens. The MEC cathode produces hydrogen gas, which is quickly converted to methane gas by H₂-oxidizing methanogens. Three 250-mL digesters were operated under continuous fed-batch mode at 40°C and fed with wastewater sludge collected from a local municipal wastewater treatment facility. The electrically-assisted digester (EAD) with the MEC reactions showed 50% removal of volatile suspended solids (VSS) as well as 60% removal of chemical oxygen demand (COD) in 6 days. However, the control digester (no MEC reactions) showed limited solids and organic destruction (only ~45% VSS removal and ~50% COD removal). The coulomb efficiency (CE) was relatively small at ~15%. This low CE result indicates that the MEC reactions made a relatively small contribution to the rapid VSS and COD destruction in 6 days. Interestingly, the MEC reactions appeared to help the rapid digestion by changing the reaction pathways for biosolids destruction with substantially stimulated fermentation. With the meaningful destruction of VSS and COD in 6 days, the electrically-assisted digester (EAD) has a potential for practical applications in wastewater biosolids treatment and management.

Electrochemically activated persulfate oxidation of recalcitrant organic compounds

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Recent interest has been placed in electrochemical advanced oxidation processes (EAOPs) using oxidants other than the hydroxyl radical to remove toxic, recalcitrant organic compounds from waste streams. Persulfate has been considered as an oxidant substitute to evaluate the effectiveness of sulfate radicals as an electrophile where hydroxyl radicals are nonreactive. Cathodic activation of persulfate was investigated in a double chambered electrochemical system in the presence of iron citrate to treat phenol, which served as a model organic compound. The advantage of oxidation reactions in the cathode is the concomitant ability to directly activate persulfate through reduction on the cathode surface itself, as well as indirect oxidation through a fenton-like reaction with persulfate and the chelated iron. Furthermore, the electrode simultaneously serves as a sustainable source for continuous Fe²⁺ regeneration and subsequent catalytic decomposition of persulfate. The efficacy of electrochemically activated persulfate on organic removal was studied considering cathode material, catholyte composition, and current density as operational parameters.

Enhancement of microbial fuel cell performance with membrane intercalating conjugated oligoelectrolytes (COEs)

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A significant obstacle for the integration of biological and electronic systems remains to be the charge transfer across the biotic-abiotic interface. Modification of this interface with conjugated oligoelectrolytes (COEs) has shown to improve charge extraction in microbial fuel cells (MFCs). COEs are small organic molecules with an electronically delocalized, hydrophobic backbone and pendant charged hydrophilic functional groups, accounting for its water-solubility and amphiphilic nature. COEs have demonstrated to spontaneously intercalate into lipid bilayers and cell membranes. Previous results have shown that COEs increase current generation in yeast, *E. coli* and wastewater systems. In wastewater MFCs, the most significant improvement was observed during start-up. In *E. coli* MFCs, COEs are not only increasing the current generation but also decreasing the internal resistance of the fuel cell, contributing to the overall increase in power density. In this poster, we present recent results to better elucidate the mechanism of action that COEs have on the performance of *E. coli* MFCs.

Large scale MFC for organic and nitrogen removal from synthetic human waste

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Several concepts for small-scale ecological decentralized waste treatment systems have been established to disseminate sanitation systems in rural environments, especially in the developing world. A large-scale microbial fuel cell (MFC) was developed for direct human waste treatment, and power production was achieved in a field deployment in Ghana. However, the field deployment revealed inefficiencies in converting waste organic matter into power. We speculate that a hierarchy of microorganisms is responsible for the degradation of complex organics present in human feces. These complex organics are broken down to simple organic compounds, such as organic acids, for which fermenters and anode-respiring bacteria compete for their use in different metabolic pathways.

In an analogous three-chamber, lab-based MFC, the breakdown of synthetic feces in the anode was explored. High-rates of COD reduction, >90%, were observed in the anode, despite minimal conversion of organic substrate to power production. The accumulation of acetic, propionic and butyric acids in a saline environment suggest the activity of halophilic fermenters. The production of biogas has also been observed, highlighting the diversion of organic compound use from anode-respiring bacteria to fermenters.

Production, separation, identification and characterization of biocompounds obtained from the operation of microbial fuel cells

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The Microbial Fuel Cell (MFC) is a technology with a capability of being used in the treatment and reduction of organic compounds, as well as for the generation of electricity in liquid effluents. The production of biocompounds in the MFC, occurs through the action of microorganisms with exoeletrogenic, metabolic and enzymatic activity, which producing metabolites and electrons, which are transported into a final electrons acceptor. Due to the potentialities of this process in the production of different metabolites, which vary according to the changes of process variables, this study proposes the separation, identification and characterization of the rheological properties of the biocompounds produced. The production of biocompounds was performed in the MFC with inoculum of marine sediment collected in the estuary located at 32°10'S; 52°5'W, and a culture medium composed of acetato, yeast extract and salts. The MFC was operated with a total volume of 2.5 L, of which 60% was allocated to the anode and 40% to the cathode, these were separated by a cation membrane. A solution of potassium ferricyanide was added to the cathode compartment as the final acceptor of the electrons released by the oxidation of organic matter in the anode compartment. The electric circuit was operated with an external resistance of 1000 Ω connected in parallel to the electrodes and the system temperature was controlled at 35°C. The downstream of the process was carried out using physical and chemical separation operations with organic solvents at a temperature of 4 °C. The obtained products were identified by infrared and magnetic resonance and the rheological characterization of the biocompound was performed using a parallel plate rheometer. It is noteworthy that the MFC process, besides being efficient in effluent treatment, as reported in the literature, can be used for the production of a compound of high added value.

Hydrogenase-independent uptake and metabolism of electrons by the archaeon *Methanococcus maripaludis* Jörg S. Deutzmann¹, Svenja T. Lohner¹, Alfred M. Spormann^{1,2}

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Direct, shuttle-free uptake of extracellular, cathode-derived electrons has been postulated as a novel mechanism of electron metabolism in some prokaryotes, and may be also involved in syntrophic electron transport between two microorganisms. Experimental proof for direct uptake of cathodic electrons has been mostly indirect and has been based on the absence of detectable concentrations of molecular hydrogen. However, hydrogen can be formed as transient intermediate abiotically at low cathodic potentials (<-414 mV) present under conditions of electromethanogenesis. Here we provide genetic evidence for hydrogen-independent uptake of extracellular electrons from a graphite cathode. Methane formation from cathodic electrons was observed in a wild type strain of the methanogenic archaeon *Methanococcus maripaludis* as well as in a hydrogenase-deletion mutant lacking all catabolic hydrogenases, indicating the presence of a hydrogenase-independent mechanism of catabolism. We also show that the presence of *M. maripaludis* cells increased the current density at cathodic potentials between -400 mV and -650 mV and, thus, effectively lowered the overpotential for cathodic electron uptake. In addition, we discovered a new route for hydrogen or formate production from cathodic electrons: Upon chemical inhibition of methanogenesis with 2-bromo-ethane sulfonate, hydrogen or formate accumulated in the bioelectrochemical cells instead of methane. These results have implications for our understanding of the diversity of microbial electron uptake and metabolism.

Enhancement of power production in Cupriavidus Basilensis microbial fuel cell using natural mediators

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Typical MFC are limited in their power production due to a high charge transfer resistance associated with low biokinetics and hindered charge mobility to the electrode. Understanding the charge transfer processes in the anode can lead to improve the energy efficiency of the MFC. In this research, the MFC was comprised of anode chamber containing a monoculture of facultative *Cupriavidus basilensis* bacterial cells growing in acetate as a sole carbon and energy source. During the MFC operation, the anode medium changes its color to green. Extraction of the green color molecules was done using two phases separation (organic and water). HPLC and MS analyses revealed that this green fraction contained pyocyanin, a redox active molecule. In this research, we examined the effect of the natural mediator on the current voltage profiles in the MFC and found evidence that addition of concentrated medium containing the pyocyanin lead to enhancement of the current and the power density of *C. basilensis* bacterial MFC.

What do exoelectrogenic microbes do in animals?

Aaron C. Ericsson¹, Daniel J. Davis¹, Catherine E. Hagan¹

Some bacteria found in the intestinal microbiota of animals are exoelectrogens. Some cells in animals sense and respond to electric fields. We use microbial electrolysis cells, next-generation sequencing, microbiota transfer, microscopy, and cell culture to understand the biological importance of bi-directional interactions among animal hosts and their gut microbes. In this talk we summarize some recent key findings in our laboratories that suggest that exoelectrogens in the gut may play a key role in the development of the immune system. We believe that identifying and characterizing interactions among these bacteria and their animal hosts may illuminate aspects of microbial biology that are of mutual interest to the fields of bioenergy technology and biomedical research.

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Development of large-scale microbial fuel cells

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Direct electricity generation from biological system makes microbial fuel cells (MFCs) be one of the potential applications for bioenergy recovery from wastewater. Intensive research and publications reveal the popularity of MFCs but most of those studies only focused lab-scale reactors with limited power output. There are two aspects for MFCs regarding to large scale: volume capacity and power output. Substantial progress towards volumetric scaling-up has been reported ranging from millimeter to litter, even further to cubic-meter scale, however, it is still far away from practical pilot-scale tests. On the other side, due to weak electricity output from single MFC, energy benefits haven't been outstanding that only a few studies examined the application of electrical scaling-up in multi-cell systems. In this study, design of MFC reactor and performance with respect to volumetric capacity, treatment efficiency, and power output from both single cell and multi-cell systems are summarized and discussed.

Ionic resistance and permselectivity of ion exchange membranes

Geoffrey M. Geise^{1,2}, Harrison J. Cassady³, Michael A. Hickner^{1,3}, Bruce Logan^{2,3}

Efficient energy recovery using microbial reverse-electrodialysis cells (MRC) and other salinity gradient energy technologies such as reverse electrodialysis (RED) relies on low resistance and high permselectivity ion exchange membranes. Anion exchange membranes (AEMs) are not as well developed as their cation exchange membrane (CEM) counterparts, so we have characterized a series of AEMs based on quaternary ammonium-functionalized poly(phenylene oxide) and poly(arylene ether sulfone) backbones to better understand how polymer structure influences ion transport properties. The water content of these polymers had a profound impact on ionic resistance and permselectivity. Increases in the water volume fraction of these polymers, as the degree of functionalization and fixed charge concentration of the polymers increased, lead to a reduction in ionic resistance and permselectivity suggesting that water content must be tuned to balance resistance and permselectivity properties. Additionally, membrane resistance was sensitive to the electrolyte used. Ionic resistance of AEMs was greater when ammonium bicarbonate (AmB), which is an electrolyte of interest for salinity gradient energy applications, as opposed to sodium chloride (NaCl) solutions were used. This change in resistance can be attributed to hydrated ion size for many of the polymers considered. Some highly functionalized polymers, however, swell to a greater extent in AmB compared to NaCl, and this phenomenon mitigates the increase in ionic resistance that results from passing current through the polymer using highly hydrated and bulky bicarbonate ions as opposed smaller chloride ions.

Bioelectrochemical systems for cultivating healthful bacteria such as *Faecalibacterium prausnitzii*Juan J. Guzman, Largus T. Angenent

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Bacterial communities in the human intestine are critical for maintaining health by preventing malnutrition, infection, and disease. Faecalibacterium prausnitzii is a bacterium that is found in the human large intestine, and that has been linked to reduced incidences of inflammation, lowering chances to develop intestinal colitis and Crohn's disease. Current literature theorizes that this health attribute from F. prausnitzii is due to its elevated butyric acid production to achieve concentrations above 10mM in the gut. In addition, F. prausnitzii is capable of interacting with electrodes in bioelectrochemical systems (BESs), but requires riboflavin (vitamin B2) to shuttle electrons to the anode. Utilizing a BES, ongoing studies are focused on analyzing the potential of employing the capability of F. prausnitzii to use indirect electron transfer with an anode or anode-like compound to provide a

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growth benefit, increasing butyric acid production. Experiments have focused on using sterile two-chambered, three-electrode BESs and microbial fuel cells to understand the role electrodes can play in manipulating the metabolic byproducts and growth of *F. prausnitzii*. Studying *F. prausnitzii* in vitro can lead to developing diagnostic and therapeutic capabilities to signal and/or treat imbalances in intestinal microbiota, minimizing risks for intestinal health concerns and leading to novel approaches for bacterial community controls.

Point of use bioelectrochemical systems for removal of pharmaceutical micropollutants

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Bioelectrochemical systems have emerged as a reliable technology for treatment of environmental pollutants. Minimal maintenance, unneeded chemical addition, and smaller operating scales needed for reactor-based technologies make them advantageous for point-of-use treatment at household and community levels. The use of a dual chambered bioelectrochemical cell was explored for point of use treatment for the removal of pharmaceutical micropollutants. Oxidation of organics in domestic wastewater at a bio-anode was coupled to cathodic reduction of oxygen to hydrogen peroxide using an open air gas diffusion cathode. Highly oxidizing hydroxyl radicals were produced using UV/H_2O_2 in situ using a medium pressure lamp. In this study, we looked at the removal of 4 model compounds: atenolol, diclofenac, carbamazepine and ibuprofen. The effect of operational parameters such as hydraulic residence time, applied voltage, and the influence of the catholyte composition were studied in depth. (Bio-)electrochemically produced H_2O_2 , activated by UV, may be an interesting route for water treatment before re-use as for domestic garden irrigation.

Biofilm engineering: measuring and manipulating bacterial communities

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Biofilms comprise the major active component of most microbial electrochemical systems. Critical length scales of transport into, out of, and within these interface-associated bacterial communities play a significant role in limiting the performance of various microbial energy conversion technologies. For the purposes of measurement and modeling, biofilms can be characterized as heterogenous, and even dynamic, material films. With this principle in mind, we present here results on engineering the structure of multispecies pathogenic biofilms based on substrate-induced modification of interspecies signaling pathways and developing methods for characterizing critical length scales of charge and mass transport in microbial electrochemical cells. Through coupling techniques of biofilm characterization, electrochemical measurements, and transport modeling, these experiments provide potential routes to controlling various characteristics of bacterial biofilm formation and improving microbial electrochemical technology performance.

The effect of sulfate reducing bacteria on energy recovery in microbial electrolysis cells

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The majority of nutrients in wastewater treatment (80% of nitrogen and 50% phosphorus) come from human urine. Nutrient removal is expensive in conventional wastewater treatment, since nitrification in activated sludge processes requires high energy demand for pumping and aeration. Additionally, phosphorus removal requires the

use of chemicals such as ferric or aluminium salts. Thus, separation of human urine from wastewater can greatly reduce the costs for nutrient removal in wastewater treatment. The high nutrient concentration in source separated urine also allows recovery of nutrients in the form of gaseous ammonia or struvite salts to be used for industrial and agricultural purposes. In addition to these benefits, human urine has a high concentration of soluble organics and high ionic conductivity. Thus, microbial electrolysis cells (MECs) can be an ideal method for urine treatment, allowing high energy recovery with minimized Ohmic losses. One challenge in applying the MEC technology for urine treatment is the relatively high sulfate concentration that resulted in substantial growth of sulfate reducing bacteria (SRB). H₂-oxidizing SRB rapidly consume hydrogen gas produced at the MEC cathode, resulting in very low biogas recovery. Acetate-oxidizing SRB compete with exoelectrogenic bacteria for organic substrates, resulting in low Coulombic efficiencies. Since both types of SRB are ubiquitous in anaerobic environments, it is difficult to exclude their activities in human urine treatment. This presentation will discuss the effects of SRB on the energy recovery and Coulombic efficiency under various MEC operating conditions.

Biochar as a sustainable electrode material for electricity production in microbial fuel cells

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This presentation introduces a newly developed wood-based biochars for microbial fuel cell electrodes and other wastewater treatment processes, which carries goo potential to significantly reduce cost and carbon footprint. In the MFC study, the biochar was made using forestry residue (BCc) and compressed milling residue (BCp). Side-by-side comparison show the specific area of BCp ($469.9 \text{ m}^2 \text{ g}^{-1}$) and BCc ($428.6 \text{ cm}^2 \text{ g}^{-1}$) is lower than granular activated carbon (GAC) ($1247.8 \text{ m}^2 \text{ g}^{-1}$) but higher than graphite granule (GG) ($0.44 \text{ m}^2 \text{ g}^{-1}$). Both biochars showed power outputs of $532 \pm 18 \text{ mW m}^{-2}$ (BCp) and $457 \pm 20 \text{ mW m}^{-2}$ (BCc), comparable with GAC ($674 \pm 10 \text{ mW m}^{-2}$) and GG ($566 \pm 5 \text{ mW m}^{-2}$). However, lower material expenses made their power output cost 17-35 US\$ Watt⁻¹, 90% cheaper than GAC ($402 \text{ US}\$ \text{ Watt}^{-1}$) or GG ($392 \text{ US}\$ \text{ Watt}^{-1}$). Biochar from waste also reduced the energy and carbon footprint associated with electrode manufacturing and the disposal of which could have additional agronomic benefits.

High power generation and pressure tolerance of activated carbon/carbon cloth cathodes for use in microbial fuel cells

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Many MFC reactor configurations are limited by cathode performance and cost. Recently, metal mesh/metal foam activated carbon cathodes have been developed for use in scaling-up MFCs. However, studies have shown that these materials will corrode under certain conditions. As a result, there is a need to develop activated carbon catalyzed cathodes with base materials that are corrosion resistant. In this talk we describe a corrosion resistant activated carbon/carbon cloth cathode with a high pressure tolerance for use in scaling-up MFCs. Maximum power density reached 3.29 W m⁻² in CEA-MFCs and 2.15 W m⁻² in cube-MFCs which is more than 2.5 times and 1.5 times higher, respectively, than that typically reported for single-chamber MFCs using platinum cathodes. In addition, the power densities reported here for CEA-MFCs are more than 200%, and in MFCs as much as 98%, greater than activated carbon cathodes containing different metal mesh/metal foam base materials reported in the literature. Static pressure head measurements show that the activated carbon/carbon cloth cathode has a pressure tolerance greater than 1.8 m, making the cathode suitable for a variety of design configurations. Finally, through example we illustrate that the activated carbon carbon/carbon cloth cathode can be produced at a cost comparable to metal mesh/foam activated carbon cathodes. These results demonstrate that activated carbon/carbon cloth cathodes are capable of achieving high performance at a low cost with no potential for corrosion, making them ideal for use in scaling-up MFCs.

Nitrate-reducing activity of anode-reducing *Geobacter metallireducens* biofilms grown in the absence of nitrate <u>Hiroyuki Kashima</u>¹, John M. Regan²

Metabolic capabilities of exoelectrogenic biofilms are of interest to develop reliable bioelectrochemical processes. Facultative nitrate reduction by some anode-reducing bacteria has been reported as a competitive metabolism to anode reduction. Based on our previous work, in which we found a very quick metabolic shift from anode reduction to nitrate reduction by *Geobacter metallireducens*, an exoelectrogenic nitrate reducer, we hypothesized that anode-reducing *G. metallireducens* cells express genes and enzymes for nitrate respiration even in the absence of nitrate. In nitrate spike tests, *G. metallireducens* biofilms reducing graphite anodes poised at different anode potentials started nitrate reduction within 30 mins after the nitrate spike, which was much quicker than the switch from ferric reduction to nitrate reduction by the same species. In addition, preliminary tests showed that cells from anode-reducing biofilms had higher nitrate reductase activity relative to ferric ion-reducing suspended cells with an in-vitro nitrate reductase activity assay. Further tests including nitrate reductase gene expression analysis will be conducted to investigate this metabolic feature seen in anode-reducing *G. metallireducens* biofilms.

Anaerobic electrochemical membrane bioreactor (ANEMBR) for treatment of low-strength organic solution with resource recovery

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This study successfully demonstrated the proof-of-concept for a novel anaerobic process that integrates a microbial electrolysis cell with a membrane bioreactor for treatment of a low strength organic solution with resource recovery (energy and reclaimed water). This new system is called an anaerobic electrochemical membrane bioreactor (AnEMBR). Nickel-based hollow fiber membranes served the dual purpose as the cathode for hydrogen evolution reaction (HER) and the membrane for filtration of the effluent. The AnEMBR system was operated for 70 days with synthetic acetate solution having a chemical oxygen demand (COD) of 320 mg/L. Removal of COD was > 95% at all applied voltages tested. Energy was recovered in the form of biogas composed predominantly of methane (83 % CH_4 ; < 1% H_2) that was generated via hydrogenotrophic methanogenesis. The net energy required to operate the AnEMBR system at an applied voltage of 0.7 V was significantly less (0.27 kWh/m³) than that typically needed for wastewater treatment using aerobic membrane bioreactors (1-2 kWh/m³). A combination of factors (hydrogen bubble formation, low cathode potential and localized high pH at the cathode surface) contributed to reduced membrane fouling in the AnEMBR compared to the control reactor (open circuit voltage).

Kinetic study of microbial hydrolysis and anode respiration in microbial electrochemical cells (MXCs) fed with primary sludge

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The use of anaerobic treatment technology has been recommended for energy recovery from wastes, especially for high-strength organic waste streams such as primary sludge (PS). Microbial electrochemical cells (MXCs) have

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advantages of i) treating high strength wastes without the need for aeration and ii) electrochemical synthesis of valuable products (e.g., H₂O₂ production at the cathode). As a step towards scaling up MXCs, we need to understand the kinetics of microbial hydrolysis of organic solids and the impacts on anode respiration. In this study, we compared direct addition of PS to a microbial electrolysis cells (MECs) versus having a prehydrolysis/fermentation step. The MECs had carbon fiber anodes with a planar area of 200 cm² in a 450-mL anode chamber. Pre-fermentation reactors were 180 mL and operated at 3-day hydraulic and solids retention times (HRT=SRT). 12% of the Total Chemical Oxygen Demand (TCOD) was recovered as volatile fatty acids in the prefermenter centrate that was subsequently fed to an MEC. On the basis of the initial PS TCOD, the MEC fed the prefermented PS recovered 12% of the TCOD as electrical current in the first 2.5 days, and conversion was negligible after 2.5 days. The maximum current density and Coulombic efficiency were 185 A/m³ and 95%, respectively. For the MEC fed PS without pre-fermentation, only 11% of the TCOD was recovered as current in the first 2.5 days, but 55% was recovered in 30 days. The maximum current density and Coulombic efficiency were 98 A/m³ and 70%, respectively. Thus, pre-fermentation improved the current density and Coulombic efficiency by producing VFAs, but the overall TCOD conversion was poor in comparison to the MEC fed PS without pre-fermentation. While hydrolysis limited the kinetics of current production without pre-fermentation, direct addition of PS led to greater conversion to electrical current than having the pre-hydrolysis/fermentation step.

Establishing a core microbiome in acetate-fed microbial fuel cells

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Establishing a core microbiome is the first step in understanding and subsequently optimizing microbial interactions in anodic biofilms of microbial fuel cells (MFCs) for increased power, efficiency, and decreased start-up times. In the present study, we used 454 pyrosequencing to demonstrate that a core anodic community would consistently develop in similar patterns over a period of 4 years given similar conditions. Results suggest a stable core microbiome in acetate-fed MFCs on both phylogenetic and functional levels, though small differences in community structure were noted between reactor designs. The core members present in all acetate-fed high-power generating biofilms were *Geobacter*, *Aminiphilus*, *Sedimentibacter*, *Acetoanaerobium*, and *Spirochaeta*, accounting for 72 ± 9 % of all genera. Variation in genetic composition and metabolic activity of the core community due to the use of alternate substrates was also explored highlighting complex interactions within the biofilms.

Improving effluent quality and accomplishing nitrogen removal in membrane bioelectrochemical reactor (MBER) Jian Li, Zhen He*

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Microbial Fuel Cell (MFC) provides a new method for wastewater treatment for less energy consumption needed for achieving effluent discharging requirement. To further improve the effluent quality, ultrafiltration hollow-fiber membranes were integrated into MFCs to form membrane bioelectrocheimical reactors (MBER). Laboratory studies of a MBER operated for more than 200 days showed that more 50% total chemical oxygen demand (COD) was removed from acetate solution and coulombic efficiency reached 30-36%. The MBER maintained 90% COD removal when treating domestic wastewater and the turbidity of the effluent was < 1NTU. This proof of concept demonstrated the feasibility of combining commercial ultrafiltration membrane with MFC technology. However, the rapid biofouling and frequent backwash created additional challenges for further development of this MBER system. To alleviate biofouling problem, an advanced configuration of inserting hollow fiber membranes into the cathode was examined in the subsequent study. During 160 days operation, more than 90% COD removal was achieved and 69% total inorganic nitrogen was removed. The turbidity of membrane permeate was below 2 NTU. Energy analysis showed that the energy recovery of 0.09kWh/m3 was significantly lower than energy demand in the MBER. To improve nitrogen removal, the MBER was modified by replacing cation exchange membrane with anion exchange membrane, and nitrogen is expected to be removed through nitrification in the anode and denitrification in the cation (nitrate migration through anion exchange membrane).

Bioelectrochemically enhanced remediation of petroleum hydrocarbons in the subsurface matrix

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This study demonstrates that electrodes in a bioelectrochemical system (BES) can potentially serve as a non-exhaustible electron acceptor for *in situ* bioremediation of hydrocarbon contaminated soil. The deployment of BES not only eliminates aeration or supplement of electron acceptors as in contemporary bioremediation, but also significantly shortens the remediation period and produces sustainable electricity. More interestingly, the study reveals that microbial metabolism and community structure distinctively respond to the bioelectrochemically enhanced remediation. Both lab (2 L) and pilot (50 L) scale BESs showed higher TPH degradation rate than control reactors, with a range of improvement between of 53-247%, which can translate to significant reduction in remediation period. The radius of influence (ROI) in TPH removal increased from 53–68 cm to 263–300 cm within 45 days in the pilot reactor with an electrode radius (ROE) of 7.5 cm, accompanied with 70.0–87.2% of TPH removal across the whole saturated reactor. The current production correlated with the TPH removal, and the maximum current output was 70.4 \pm 0.2 mA/m² for the pilot BECS, and 73-86 mA/m² for lab studies. Comprehensive microbial and chemical characterizations and statistical analyses indicated strong correlations between TPH and microbial enzyme activities, as well as soil pH and conductivity. Distinctive microbial communities were identified at the anode, in soil with electrodes, and soil without electrodes.

Proton diffusion limitations in thermophilic microbial electrochemical cells using *Thermincola ferriacetica* as an anode respiring bacterium

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The effects of temperature, buffer concentration, and pH on current production by Thermincola ferriacetica, a thermophilic, gram positive, anode respiring bacterium (ARB), in microbial electrochemical cells (MXCs) were assessed. Proton diffusion is hypothesized to be a major limitation for current production in MXCs. Since increased temperatures and buffer concentrations enhance the diffusion of protons out of the biofilm, we postulated that MXCs operated with T. ferriacetica will produce more current than mesophilic monoculture MXCs at similar buffer concentrations. H-type MXCs were operated continuously with 10 mM, 25 mM, 50 mM, and 100 mM bicarbonate buffer and 25 mM Acetate at 60°C with T. ferriacetica. Reactors were then operated in batch mode and pH was adjusted using NaOH. Anode potential was poised at -0.06V vs SHE. Maximum current density in MXCs operated with 10mM bicarbonate buffer generated 6.8±1.1A m⁻², which is higher than MXCs operated at 30°C using G. sulfurreducens (~4.0A m⁻²) at the same buffer concentration. Increasing buffer concentrations in thermophilic MXCs from 10 mM to 100 mM bicarbonate increased current density by 40±6%; resulting in a maximum current density of 11.2±2.7A m⁻². Adjusting pH between 6.7 and 8.0 showed a linear increase in current production (R²=0.9585). Cyclic voltammetry analysis and scanning electron microscopy images suggest a biofilm consisting of a complex extracellular matrix. Confocal laser scanning microscopy images indicate that T. ferriacetica biofilms have thicknesses from 50 to greater than 150 µm. Operating MXCs at 60°C resulted in enhanced current production. Increased buffer concentrations and pH also contribute to higher current production. This data supports that current production in MXCs is limited by proton diffusion in the biofilm and confirms that thermophilic MXCs can treat high temperature wastewaters, require less buffer, and produce enhanced current generation over mesophilic MXCs.

Boosting the performance of microbial electrochemical cells fed with landfill leachate using Fenton preoxidation

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Recent studies highlight the growing interest to improve the performance of microbial electrochemical cells (MXCs) fed by complex organic substrates, such as landfill leachate. Landfill leachate typically is wastewater that contains high concentrations of organics, ammonium-nitrogen (NH4 -N), and inorganic salts. It also has low biodegradability (based on the BOD5/COD ratio), which results in poor MXC performance (e.g., low current density (j), Coulombic efficiency (CE), and organics removal). We evaluated whether or not Fenton pre-oxidation of recalcitrant landfill leachate (BOD5/COD = 0.31) enhanced j, Coulombic efficiency (CE), and organics removal in a microbial electrolysis cell (MEC). We evaluated a range of operational parameters for the Fenton process, such as temperature (25–45 °C), pH value (2.5–7), and H2O2 concentrations (50 – 500 mM). During Fenton oxidation of leachate, the leachate quality changed significantly due to reduction in concentrations of organic components (i.e., COD, TOC, and phenol). At the conditions giving the largest effects (pH = 3.5, molar ratio of H2O2:Fe2+ = 4, and initial H2O2 concentration 200 mM), the average COD, TOC, and phenol removals were 54%, 46% and 52%. In addition, we observed a significant increase in the biodegradability (BOD5/COD ratio) and acid metabolites in the treated leachate: from 0.31±0.10 to 0.50±0.12 and from 155±35 to 320±40 mg/L, respectively. Feeding the treated leachate to the anode of an MEC remarkably enhanced its performance: to 28-35% COD removal, 40-45% CE, and j of 1.45–1.80 A/m2, compared to 4–6% COD removal, < 1% CE, and 0.06–0.15 A/m2 for the raw leachate. This supports the value of Fenton pre- oxidation before an MEC for enhancing COD stabilization and electron recovery as current when treating a recalcitrant wastewater like leachate.

Estimation of optimal electrode size for sediment microbial fuel cell

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Sediment microbial fuel cell (SMFC) is a device used as an alternative power source for running aquatic sensors to harvest the electricity by locating the anode in sediment phase and placing the cathode in an oxygen-rich water phase because they generate energy by oxygen reduction reaction in water and converting chemical energy of organic matters in sediment to electric energy. Energy generated by SMFC can be increased as electrode area increase, but electrode materials forming mainly the SMFC are not economically feasible because of cost issue. For this reason, electrode size is a crucial factor affecting electric energy generation and installation cost. However, still there are no reports relative to determination of optimal size for SMFCs due to different sediment environments. Herein, we propose a method to determine electrode size for SMFCs by using a correlation of electrode surface area and current density, which can be applied to various sediment environments. Current could be proportional to its electrode size at constant overpotential. To induce the correlation between electrode size, current, and overpotential, we determined proportionally increased anode and cathode size in same ratio and operated SMFCs in more than 500 days. As a result, the optimal electrode size was calculated for a sensor running even durational performance change. The goal of this study was to suggest a model that can determine electrode size regardless of environmental change to operate aquatic sensors.

Multi-variable modeling for microbial fuel cell and cathode mass transport

Shiqi Ou¹, Hiroyuki Kashima², Yi Zhao², Jay Regan², Matthew M. Mench^{1,3}

A series of computational models was built for an air-cathode microbial fuel cell (MFC) system to better understand the interplay of the biological, chemical, and electrochemical reactions coupled with mass and electron transport. This study divided the work into three submodels - anode half-cell, cathode half-cell, and full cell - so that the various parameters and reactions at each electrode and their interrelatedness could be analyzed. The anode transient model was formulated based largely on Picioreanu et al's biofilm model, with anode biofilm growth and acetate concentration predicted by this simulation. The cathode model was built to compare the oxygen mass transfer in cathode materials and also competition in the cathode biofilm between cathode-oxidizing autotrophic bacteria and heterotrophic bacteria. Through the formulated model, the oxygen reduction reaction (ORR) occurs in both the catalyst layer and the cathode biofilm, with hydroxide production by the cathodic ORR and the exoelectrotrophic autotrophic bacteria in the biofilm. The impact of facilitated mass transport observed experimentally with heliox compared to standard air mixture was examined in detail with this model. The full-cell simulation combines the submodels with ohmic losses and predicts polarization curves and power density of the full MFC. Model data will be validated using experimental data as well.

Dual stage bacteriological triacylgylceride accumulating system

Nikita Panov¹ and John M. Pisciotta^{1,2}

Global demand for sustainable fuels, like biodiesel, is increasing and there is a growing need for sustainable, non-food based triacylglyceride (TAG) feedstocks. Growth of lipid rich algae is one avenue but phototrophs cannot be grown at night. Here we describe a dual stage, bacteriological system capable of processing acetate-rich wastes into bacterial triacylglyceride (TAG) for sustained biofuel production. In stage 1, the gram negative exo-electrogen, Geobacter sulfurreducens was cultured in Microbial Electrolysis Cells (MECs) containing synthetic waste-water with 1 g /L acetate (CH_4CO_2) to produce CO_2 and H_2 gas. This gas was passed through a one-way valve into a second, aerobic chamber, in which the TAG accumulating bacterium Rhodococcus opacus strain PD630 was cultivated in 20 mM PBS media. CO_2 and H_2 gasses served as carbon and energy sources for autotrophic TAG production. This system was compared to a control in which R. opacus PD630 was grown exclusively on acetate as well as a mixotrophic embodiment. The utility of this dual stage bioreactor is that it could be used to produce TAG from wastewater treatment during the day and night.

Sustainable bioelectrochemical desalination: challenges and development

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In a microbial desalination cell, ion exchange membranes serve a key role in creating different chambers and separating the targeting ions from salt solution. Fouling on the membranes is a major concern in MDC regarding the desalination performance, the contamination to desalinated water and its own maintenance. The relatively large inter-membrane distance of MDC compared to electrodialysis, also affect the desalination performance, while might alleviate fouling effect and decrease fabrication complexity. In this presentation, we summarize the findings on MDC in our work. We evaluated the membrane behavior in MDCs by an over eight-month study to see how ion-exchange membranes were deteriorated by biofouling/scaling with actual wastewater and synthetic seawater. Since small inter- membrane distances and long HRTs cannot be achieved simultaneously at a fixed water production rate, we further investigated the performance of MDC with different inter-membrane distances (0.3 -

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2.5 cm). To improve the flexibility of MDC construction and operation, we also developed a new configuration with decoupled anode and cathode, which makes it possible to adjust the ratio of liquid volumes between different compartments in a more convenient way than in the previous MDCs. Easier repair/replacement of the problematic units can be achieved without significantly modifying the whole MDC system.

Influence of electricity generation on reverse salt flux in an osmotic microbial fuel cell Mohan Qin, Zhen He*

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Osmotic microbial fuel cell (OsMFC) is an emerging concept which integrates forward osmosis (FO) and microbial fuel cell (MFC). Previous studies have demonstrated that using FO membrane as a separator can extract water from wastewater and generate more electricity. But in FO membrane, the reverse salt diffusion from draw solution to feed solution could reduce driving force for water transport and may contaminate the feed solution. Compared to FO process, the reverse salt diffusion would be affected by electricity generation in OsMFC. In this study, the influence of electricity generation on the reverse salt flux in OsMFC was investigated. We also explored the influence of reverse salt diffusion on the water flux and microbial community in OsMFC.

Characterization of acid mine drainage-derived biocathode communities

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Bioprospecting was performed using acid mine drainage (AMD)-sediments with the goal of exploring the development of novel biocathodes that include acidotolerant or acidophilic exoelectrotrophic populations. Three sediment microbial fuel cell (SMFC) configurations were used to enrich microbial communities from the sediments on aerobic graphite fiber brush cathodes: (i) AMD-Winogradsky columns using small (2.5-cm length) brushes (operated 10 months), (ii) AMD-Winogradsky columns using large (7-cm length) brushes (operated 1 month), and (iii) AMD-SMFCs inoculated from the columns (operated 3 months). The cathodes from these systems were shown by LSV and EIS to be biocatalytically active, with significantly lower charge transfer resistance than abiotic and gamma-irradiated cathodes. Duplicate samples from each cathode type were pyrosequenced to characterize the Bacterial and Archaeal communities based on 16S rRNA gene sequences. Some Archaeal amplications failed and very few sequences were retrieved from those that generated PCR products, so the Archaeal communities were poorly characterized and perhaps numerically scarce relative to Bacteria. Cathodes from the small-brush Winogradsky columns were dominated by Firmicutes (52 and 93% of sequences), whereas the large-brush cathodes showed an overwhelming dominance of Proteobacteria (95 and 99%), the reinoculated reactors (iii) showed a predominance of Proteobacteria (71 and 84%) and Actinobacteria (13 and 22%) sequences. The Proteobacteria in the Winogradsky columns using large brush biocathodes were primarily Acidiphilium (Alphaproteobacteria) or Acidithiobacillus from the Gammaproteobacteria. The Proteobacteria from the reinoculated reactors were primarily Gammaproteobacteria, of the order Xanthomonadales or unclassified, or Acidiphilium from the Alphaproteobacteria. While the specific contributions of these populations to the biocathodic performance are not known, these communities present interesting assemblages with low pH biocathodic activities.

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Competition for electron donors in anode-respiring biofilms

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Waste streams fed to bioelectrochemical systems are complex and the anode is not the only acceptor available to the microorganisms. A poorly understood phenomenon in MFCs is the role of competition between microbial communities that can transfer the electrons to an anode and microorganisms that use other electron acceptors naturally present in waste. Competition in microbial communities could significantly impact power produced. Nitrogen species are a major constituent of wastewater and nitrate can act as an electron sink which could adversely affect power production. Different environmentally relevant nitrate concentrations, 5, 10 and 20 mg-N/L, were introduced to the anode compartment of operating MFCs. Experiments were performed in both electron donor limiting and non-limiting conditions. The COD removal did not change significantly due to the presence of nitrate in both conditions. However, polarization studies performed over the course of the experiment suggest that the maximum power density decreased with increasing concentrations of nitrate. The time-dependent changes in anode microbial community structure over time are being examined by sampling using denaturing gradient gel electrophoresis (DGGE) and molecular sequencing techniques.

Applications of a larger-scale MFC for wastewater treatment and power production

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Microbial fuel cells (MFCs) use exoelectrogenic bacteria to break down organic matter and generate electricity as well as treat wastewater. MFCs can vary in size and configuration, but many studies utilize a smaller reactor body with either one or two chambers. Studies using larger scale reactors are necessary to improve system efficiency, performance, and to understand chemical oxygen demand (COD) removal kinetics before implementation in domestic wastewater treatment facilities. Such a technology could serve as a practical method for wastewater treatment while also offsetting energy costs at wastewater treatment facilities. In this presentation we will summarize the performance results of a larger-scale, single chamber, multi-brush anode MFC operated with wastewater under batch and continuous flow conditions. Performance was evaluated based on power output, COD removal, and removal kinetics.

A novel technique for the development of microbial biofilms exhibiting biocathodic activity Rosanna Stoutenburg¹, Doug Call²

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The chemically-catalyzed cathodes typically employed in microbial fuel cells (MFCs) often utilize materials that foul over time, introduce pollutants, and are economically prohibitive. A promising alternative to chemical cathodes are microbial biocathodes that do not require expensive or toxic catalysts and are capable of accepting electrons from bioanodes. To expand biocathode applicability and advance their efficiencies, new methods of developing biocathodic biofilms are of great necessity. This study developed biocathodic microbial communities using a wastewater-inoculated bioanode to biocathode conversion strategy. Bioanodes were established in reactors operated at -0.15 V or +0.15 V (versus SHE) on either acetate or formate. After 20 days of operation, all the reactors exhibited consistent peaks in electric current characteristic of batch mode cycles where the acetate-fed +0.15 V reactors produced the greatest current maxima. Within 30 days, cathodic cyclic voltammetry revealed that the formate-fed reactors produced larger cathodic current than the acetate-fed reactors and abiotic control. In the absence of substrate, linear sweep voltammetry confirmed that formate-enriched biocathodes generated larger cathodic current with, the highest current produced by the formate-enriched biofilms acclimated at +0.15 V. Unlike the formate-fed reactors, acetate-fed reactors did not exhibit electron consumption during 2 hours of cathodic polarization in the absence of substrate. A subsequent 2 hour anode polarization of the formate-fed reactors

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yielded an average electron release of $105\% \pm 28\%$ of the electrons consumed during the cathodic polarization suggesting electron accumulation at or near the electrode-bound biofilms. These results indicate that the degree of biocathodic activity exhibited by enriched bioanodes is substrate-dependent and that formate-fed bioanodes yield microbial communities having greater biocathodic activities than those on acetate. [Poster presentation]

Rate of individual nutrient separation in bioelectrochemical urine treatment

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Human urine is a large contributor of nutrients and soluble organic matter in domestic wastewater. The separation of urine at the source offers a potentially promising method for energy and nutrient recovery. The high ionic conductivity and relatively alkaline pH of hydrolyzed human urine provide an ideal condition for bioelectrochemical treatment using exoelectrogenic microorganisms. Exoelectrogenic microorganisms at the anode can oxidize organic substrates in human urine and the energy released can be used to separate nutrient ions from human urine. Separation of nutrient ions (NH₄⁺ and HPO₄²⁻) from 1:10 diluted synthetic human urine was demonstrated in this study. An ion exchange membrane (IEM) stack was constructed in a lab-scale microbial electrolysis cell (MEC) similar to microbial desalination cells (MDCs). Unlike MDC studies, one of our important objectives was to look into the competitive separation of nutrient ions versus other common ions in human urine. Ionic conductivity, ammonium concentration, and phosphate concentration were monitored in laboratory experiments with the constructed reactor. The rate of ammonium separation was consistently faster than the rate of conductivity increase in the concentrate (urine solution that collects ions in the IEM stack), indicating that ammonium ions are favorably separated through cation exchange membranes compared to the other common cation (i.e., sodium). In contrast, separation of phosphate was slow compared to the other major anions in urine (chloride and sulfate). The equivalent ionic conductivity of individual ionic species was found to have a significant impact on the rate of separation of nutrient ions, explaining the rapid separation of ammonium ions and slow concentration of phosphate. In IEM-based processes for nutrient recovery from source separated human urine, we recommend that future studies focus on enhancing selective phosphate separation, for example, by developing specialized anion exchange membranes selective for phosphate.

Comparison of complex and ideal effluents in different bench scale microbial electrolysis cell architectures Mark L. Ullery and Bruce Logan

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The performance and efficiency of microbial electrolysis cells (MECs) for concurrently treating wastewater and recovering energy is dependent on substrate conditions. High strength waste streams, including industrial and fermentation effluents, have shown greater potential for energy recovery in MECs than domestic wastewater sources, but are widely variable and process dependent. Typical wastewater evaluation tools, including COD and BOD, are not directly indicative of substrate performance in MEC reactors. Cheap, high through-put mini MEC reactors have been used previously to evaluate treatment performance in a wide range of effluents, but they have not been directly compared to larger bench scale reactor architectures. In this presentation we summarize performance data taken in both mini and cubic MEC reactors with various complex and ideal substrates to provide a better understanding of the relationship between these architectures and their utility for evaluating treatment performance with complex effluents.

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Anion exchange resin in a microbial reverse electrodialysis cell (MREC) reduces nitrogen crossover Maxwell J. Wallack¹, Marta C. Hatzell², Bruce E. Logan³

Microbial fuel cells (MFCs) are an emerging option for simultaneous wastewater treatment and energy recovery. Exoelectrogenic bacteria in an MFC oxidize wastewater organics and release electrons to a solid conductor. If coupled to a reduction reaction through an external circuit, electricity is generated. However, MFCs suffer from low power. One promising way to increase power is by coupling an MFC with reverse electrodialysis (RED). RED captures energy through the controlled mixing of two salt solutions of different concentration. Mixing is controlled by separating solutions by a series of alternating cation (CEM) and anion (AEM) exchange membranes. Studies show this increases MFC power by ~5X^[1]. Further power increases in MFC technology can make them a cost-effective treatment option.

In a microbial based RED system (MREC), the AEM located next to the anode chamber allows for unwanted nitrogen crossover from high concentration ammonium bicarbonate (AmB) solution^[2]. High nitrogen concentrations in the anode chamber have the potential to reduce power density and kill the microbial community. To reduce this effect, an extra low-concentration chamber and additional AEM can be added next to the anode. However, the additional low-concentration chamber adds resistance and performs poorly. It is hypothesized here that filling the end chamber with anion-exchange resin can decrease both the resistance and nitrogen crossover.

An experimental setup to obtain stack resistances used an RED stack with high and low concentrations of NaCl (1 M and 10 mM) and platinum anodes and cathodes. The anode chamber was filled with a sodium acetate and phosphate buffer solution, and cathode with 1 M NaCl. Anion exchange resin in the extra chamber decreased stack resistance from 109 to 83 Ω , in line with the original RED stack. Continuing work is set to investigate the role resin has in mitigating nitrogen crossover.

Extraction and re-immobilization of exoelectrogenic biofilms

Hao Wang ¹, Rosanna Stoutenburg ², Douglas F. Call ³

There is great interest in using microbial electrochemical technologies to degrade organic materials in wastewater as well as generate electrical current. One limiting factor in applying these systems at large scales is the slow development of exoelectrogenic biofilms on the electrodes. Recent studies of pilot-scale reactors requiring extended startup periods highlight gaps in our understanding of colonization and competition among exoelectrogenic and non-exoelectrogenic bacteria when using real wastewaters. As an alternative to in situ biofilm growth on microbial fuel cell (MFC) anodes, we are examining a new approach of extracting and re-immobilizing the existing biofilms to sterile anodes. We are currently exploring several biocompatible immobilizing agents and their abilities to extract intact and viable cells. Our initial results suggest that biofilm immobilization, extraction and re-application can lead to a substantial decrease in MFC startup time relative to wastewater-inoculated controls. Our future work in this area will be addressing the adhesive strength of these re-immobilized biofilms and determining their feasibilities in flow-through systems which are typical in most wastewater treatment processes.

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Bioelectrochemical platform for sustainable environmental remediation and energy generation

Heming Wang¹, Haiping Luo², Paul H. Fallgren³, Song Jin³, and Zhiyong Jason Ren¹

The increasing awareness of the energy-environment nexus is compelling the development of technologies that reduce environmental impacts during energy production as well as energy consumption during environmental remediation. Countries spend billions in pollution cleanup projects, and new technologies with low energy and chemical consumption are needed for sustainable remediation practice. This perspective review provides a comprehensive summary on the mechanisms of the new bioelectrochemical system (BES) platform technology for efficient and low cost remediation, including petroleum hydrocarbons, chlorinated solvents, perchlorate, azo dyes, and metals, and it also discusses the potential new uses of BES approach for some emerging contaminants remediation, such as carbon dioxide in air and nutrients and micropollutants in water. The unique feature of BES for environmental remediation is the use of electrodes as non-exhaustible electron acceptors, or even donors, for contaminant degradation, which requires minimum energy or chemicals but instead produces sustainable energy for monitoring and other onsite uses. BES provides both oxidation (anode) and reduction (cathode) reactions that integrate microbial-electro-chemical removal mechanisms, so complex contaminants with different characteristics can be removed. We believe the BES platform carries great potential for sustainable remediation and hope this perspective provides background and insights for future research and development.

Hydrogen production from fermentation effluent using a continuous flow microbial reverse-electrodialysis electrolysis cell

<u>Valerie Watson</u>¹, Marta Hatzell², Bruce Logan¹

Fermentation of renewable biomass is a promising technology for sustainable hydrogen production; however, the process is hindered by the accumulation of high strength waste streams. In this study we used a continuous flow microbial reverse-electrodialysis electrolysis cell (MREC) with no added electrical grid energy to produce additional hydrogen using fermentation effluent (provided by the National Renewable Energy Lab) as the substrate. The anolyte had a total COD of 1.1 - 1.2 g/L, and consisted of the fermentation effluent diluted with 100 mM sodium bicarbonate buffer. The system was tested with the anolyte hydraulic retention times (HRT) set at 24, 12 and 8 hours. An HRT of 8 h provided the most stable anode potential throughout the reverse-electrodialysis stack cycles and provided an increase in hydrogen production. Hydrogen was produced from this system at a maximum rate of $0.9 + - 0.1 L H_2/L$ - reactor/day (HRT = 8hr). The percentage of COD removed by the MREC decreased from 73% to 60% with decreased HRT, but the rate of COD treated increased from 0.8 to 1.9 g/L/d. Hydrogen production at the cathode of the MREC increased from 0.3 to 0.9 L/L-reactor/d with decreased HRT. Hydrogen yields also increased from 0.8 to 1.0 L/g COD with the decrease in HRT.

Characterization of biological nanowires in *Geobacter sulfurreducens* as a conductive material Hengjing Yan¹, Guillermo C. Bazan²

Metal-reducing bacteria *Geobacter sulfurreducens* have been found to be able to transfer electrons to external electron acceptors (EEA) such as insoluble Fe(III) oxides, or anode electrodes in bioelectrochemical systems for electricity production, by either direct cell-EEA contact or the production of type IV pili as biological nanowires. The

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biological nanowires in *G. sulfurreducens* have been reported to be electrically conductive with and without bacteria cells and enable electron transfer from distant *G. sulfurreducens* cells in biofilm to electrodes.

However, up to now, the conducting mechanism of nanowires in G. sulfurreducens is still not clear. Previous scanning tunneling microscopy results did not find the evidence of cytochrome heme groups contributing to nanowires' conductivity. Denaturing cytochromes did not affect nanowire conductivity of G. sulfurreducens either. Although the protein sequence suggested less than 9% aromatic amino acids content in nanowire's protein pilin, PilA, X-ray diffraction patterns of purified nanowires surprisingly indicated tightly packed crystalline regions against amorphous background, leading to the guess of π - π interchain stacking between aromatic amino acids present in nanowires. In this presentation, we will show the characterization of the material properties of nanowires in G. sulfurreducens regarding their electric conductivity and elasticity under different conditions. Further exploration of their conducting features and the feasibility of using biological nanowires as conductive material will also be covered.

Poly(vinylidene fluoride-co-hexafluoropropylene) phase inversion coating as a diffusion layer to enhance the cathode performance in microbial fuel cells

Wulin Yang¹, Fang Zhang¹, Weihua He², Jia Liu¹, Michael A. Hickner³ and Bruce E. Logan^{*1}

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A low cost poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) phase inversion coating was developed as a cathode diffusion layer to enhance the performance of microbial fuel cells (MFCs). A maximum power density of $1430 \pm 90 \text{ mW m}^{-2}$ was achieved at a PVDF-HFP loading of 7.1 mg cm⁻² (4:1 polymer:carbon black), with activated carbon as the oxygen reduction cathode catalyst. This power density was 31% higher than that obtained with a more conventional platinum (Pt) catalyst on carbon cloth (Pt/C) cathode with a poly(tetrafluoroethylene) (PTFE) diffusion layer (1090 \pm 30 mW m⁻²). The improved performance was due in part to a larger oxygen mass transfer coefficient of 3×10^{-3} cm s⁻¹ for the PVDF-HFP coated cathode, compared to 1.7×10^{-3} cm s⁻¹ for the carbon cloth/PTFE-based cathode. The diffusion layer was resistant to electrolyte leakage at water column heights of 41 \pm 0.5 cm (7.1 mg cm⁻² loading of 4:1 polymer:carbon black) to 70 ± 5 cm (14.3 mg cm⁻² loading of 4:1 polymer:carbon black). This new type of PVDF-HFP/carbon black diffusion layer could reduce the cost of manufacturing cathodes for MFCs and initial investment of building pilot MFC system.

Sustained cathodic hydrogen production using *Geobacter sulfurreducens* and *Methanosarcina barkeri* Matthew D. Yates*, Michael Siegert, and Bruce E. Logan

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Hydrogen is an important energy storage compound that can be used as a clean alternative to fossil fuels. However generation of hydrogen usually requires precious metal catalysts to lower the overpotential for hydrogen formation. Biocatalysts, including microorganisms, can be used an alternative to precious metals. Pure and cocultures of *Geobacter sulfurreducens* and *Methanosarcina barkeri* were investigated as biocatalysts for hydrogen production over an extended period of time (5 months). Stable hydrogen production was observed during this period (111 ± 33 nmol/ml/day; $^{2.9}$ ± $^{1.2}$ µmol/cm 2 /day) with Coulombic recoveries of 114 ± 48 %. Methane production was not observed except during the *M. barkeri* inoculation cycle. Abiotic deconstruction controls were also investigated to determine the relative importance of individual components to hydrogen production. Cell extracts of *G. sulfurreducens* and *M. barkeri* could not account for the hydrogen production exhibited, suggesting intact cells are important for the hydrogen production process. However, a mixture of *G. sulfurreducens* (8 ± 1 nmol/ml/day) and *M. barkeri* (7 ± 1 0.4 nmol/ml/day) cell extract with cysteine could account for half of the hydrogen production rate (43 ± 1 nmol/ml/day) observed in reactors inoculated with live cells. Acetate was found

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in poised reactors inoculated with live cells ($87 \pm 3 \text{ nmol/ml/day}$), but not in any abiotic control reactor. Proteins attached to the electrode surfaces were extracted and analyzed to elucidate possible mechanisms of hydrogen production. Surfaces were also modified with iron, nickel, and cobalt minerals after 5 months of incubation as determined by different spectroscopic and microscopic techniques.

Advanced electrochemical characterization of *Geobacter sulfurreducens* to reveal electron transport pathways Rachel Yoho^{1,2}, Sudeep Popat¹, César Torres^{1,3}

¹Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, USA

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Microbial electrochemical cells have not only provided an opportunity for several practical applications for sustainably, but also allowed the study of the phenomenon of extracellular electron transfer in anode-respiring bacteria. The application of advanced electrochemical techniques on these systems continues to reveal extracellular respiration properties of the model bacterium, *Geobacter sulfurreducens*. We have applied several techniques including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and chronoamperometry, focusing especially on relatively low potentials (-0.3 to 0 V vs. standard hydrogen electrode, SHE), to reveal unique features of the energy metabolism of *G. sulfurreducens*.

CV reveals the rate of extracellular respiration as the current (*j*) resulting from a pre-defined sweep of the anode potential (V), which governs the energy per electron available to the biofilm. Similar to several other studies, we have observed deviations in the experimental results from the classic sigmoidal Nernstian relationship based on the assumption of a single limiting redox process governing the overall *j*-V response. To further investigate this discrepancy, we used EIS performed over a range of discrete poised potentials, to determine resistances to electron transfer in anode biofilms. EIS results suggest the presence of more than one distinct "governing" redox processes, attributable to distinct cofactors that contribute to extracellular respiration on anodes. Additionally, chronoamperometry experiments reveal the dynamics of how the primary redox factor used can change in real time, with the biofilm exhibiting different behaviors depending on the anode potential.

Our results suggest the presence of multiple governing processes in electron transport for *Geobacter sulfurreducens*, with the ability to change rapidly for optimization. From these results, we believe that the phenomenon and the model, the Nernst-Monod relationship, need to be further investigated and expanded.

Integrating microbial desalination cells with forward osmosis to achieve sustainable water reclamation Heyang Yuan, Zhen He*

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In recent years global energy and water scarcity is encouraging the development and investigation of new treatment technologies. As one of the emerging technologies microbial desalination cell (MDC) has a promising potential for wastewater treatment in the future: simultaneous contaminant removal and water reclamation with low energy consumption. However, the drawbacks of MDCs hinder their practical application. For example, the effluent (i.e. catholyte) needs further treatment before being released into environment due to its increased salinity. To diminish salt water discharge, forward osmosis (FO) was introduced in this study. During FO process diffusion of fresh water is spontaneously driven by salinity gradient across a semi-permeable membrane. By integrating these two technologies, we could achieve improved desalination and wastewater reuse.

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Novel osmotic bioelectrochemical systems for simultaneous water desalination and wastewater treatment Fei Zhang¹, Zheng Ge¹ and Zhen He¹

A novel osmotic microbial fuel cell (OsMFC) was developed by using a forward osmosis (FO) membrane as a separator. The performance of the OsMFC was examined with either NaCl solution or artificial seawater as a catholyte (draw solution). A conventional MFC with a cation exchange membrane was also operated in parallel for comparison. It was found that the OsMFC produced more electricity than the MFC in both batch operation (NaCl solution) and continuous operation (seawater), likely due to better proton transport with water flux through the FO membrane. Water flux from the anode into the cathode was clearly observed with the OsMFC but not in the MFC. The solute concentration of the catholyte affected both electricity generation and water flux. These results provide a proof of concept that an OsMFC can simultaneously accomplish wastewater treatment, water extraction (from the wastewater), and electricity generation. The potential applications of the OsMFC are proposed for either water reuse (linking to reverse osmosis for reconcentration of draw solution) or seawater desalination (connecting with microbial desalination cells for further wastewater treatment and desalination). We have further developed osmotic bioelectrochemical systems by examining the effect of draw solutes, treating actual wastewater and linking to other bioelectrochemical systems.

Improved activated carbon based air-cathodes for microbial fuel cells by chemical and physical modifications Xiaoyuan Zhang^{1*}, Deepak Pant², Fang Zhang¹, Jia Liu¹, Bruce E. Logan^{1**}

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Activated carbon (AC) is a low-cost, effective and environmentally sustainable catalyst for oxygen reduction in air-cathode microbial fuel cells (MFCs). To improve performance, AC was modified by three methods: (1) pyrolyzed with iron ethylenediaminetetraacetic acid (AC-Fe); (2) heat treated at 800 °C under argon gas (AC-Heat); and (3) mixed with 10 wt% carbon black (AC-CB). AC-Fe and AC-Heat cathodes produced similar maximum power densities in MFCs of 1410 ± 50 and 1400 ± 20 mW/m2, ~35% higher than that of the plain AC (1040 ± 20 mW/m²). Adding carbon black improved performance by ~16% (1210 ± 30 mW/m²) relative to plain AC. The power production with these different treatments was equal to or larger than that obtained with Pt (1270 ± 50 mW/m²). Most of these differences in performance were due to charge transfer resistance. After 3.5 months, the modified AC cathodes generated maximum power densities (1180 ± 130 mW/m², Fe; 1200 ± 50 mW/m², heat; and 1160 ± 60 mW/m2, CB) that were 12% to 15% higher than the plain AC (1040 ± 50 mW/m²). After 5.5 months, biofilm and salt precipitation on the AC cathodes were removed, and power densities improved by 3% ~5% compared to results after 3.5 months, indicating only a small portion of the change in power was due to reversible fouling. The Pt cathodes had very poor durability, and after 5.5 months the maximum power was only 390 ± 60 mW/m². Based on the similar performance of the modified AC cathodes, blending 10% CB was concluded to be the most cost-effective method to enhance AC performance.

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WORKSHOP PRESENTATION ABSTRACTS

Quantifying specific resistances at multiple interphases in microbial reverse electrodialysis cells using multichannel electrochemical impedance

Roland D Cusick^{1,2}, Fang Zhang², Marta C. Hatzell², and Bruce E. Logan²

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Microbial reverse electrodialysis cells (MRCs) enhance power production and treatment of wastewater by operating microbial fuel cells electrodes on either side of a reserse electrodialysis membrane stack. When current is generated in a MRC, internal resistance creates voltage drops, reducing power production. Internal resistance can be classified as solution, membrane, kinetic, diffusion and capacitive resistances. The sum of all of these resistances can be easily quantified from the slope of a linear polarization curve. To differentiate between ohmic, capacitive, diffusive and kinetic resistances in a cell, electrochemical impedance spectroscopy must be employed. In this workshop we present a novel method of simultaneously quantifying resistances at the three interphases of a MRC (anode, membrane stack, and cathode) by operating multiple galvanostatic EIS channels in a stack arrangement.

Electrochemical measurements – utilizing your hardware to the fullest

Jaroslaw Syzdek, Bill Eggers

Bio-Logic USA LLC, Knoxville, TN, USA; yarik.syzdek@bio-logic.us

Electrochemistry is a unique field of science. From chemical point of view it's unique because the energy levels of materials can be driven with a dial (literally) causing all sorts of interactions, as well as, driven in reverse, unlike any other chemical reaction. From the electrical point of view the electrochemical cell is unlike other electric circuits because it is a highly non-linear and complicated system, opposite to R, C, L or any combination thereof. Successful and meaningful electrochemical research requires knowledge of both parts of the experimental setup: the system under study and the hardware, as well as the wiring connecting the two. This talk will cover some essential information about potentiostats, how they operate and how to utilize fully their capabilities in electrochemical research.

Measurement of electron transport rates through electrochemically active biofilms

Darryl A. Boyd¹, Jeffrey S. Erickson², Jared N. Roy³, Rachel M. Snider^{2,4}, Sarah M. Strycharz-Glaven², and <u>Leonard M. Tender</u>²*

The general approach to investigate the mechanism of electron transport through a material is to place the material between two electrodes and measure the dependency of the rate of electron transport through the material from one electrode to the other, in the form of electrical current, on the potentials applied to the electrodes. Materials utilizing different mechanisms of electron transport exhibit different current-potential dependencies. Theory and methodology will described for performing biofilm electron transport rate measurements and interpreting the results. The goal of this presentation is to enable researchers to perform electron transport rate measurements on their own electrochemically active biofilms.

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^{*&}lt;u>Tender@nrl.navy.mil</u> (Authors listed alphabetically)

Reference and counter electrode positions affect electrochemical characterization of bioanodes in different bioelectrochemical systems

<u>Fang Zhang</u>, Jia Liu, Ivan Ivanov, Marta C. Hatzell, Wulin Yang, Yongtae Ahn, and Bruce E. Logan Department of Civil & Environmental Engineering, Penn State University, USA; <u>fuz105@psu.edu</u>

The placement of the reference electrode (RE) in various bioelectrochemical systems is often varied to accommodate different reactor configurations. While the effect of the RE placement is well understood from a strictly electrochemistry perspective, when the bioanode is used as the working electrode (WE), there are impacts on exoelectrogenic biofilms in engineered systems that have not been adequately addressed. Increased distances between the RE and counter electrode (CE) in microbial fuel cells (MFCs) can alter bioanode characteristics through the acclimation to different anode potentials. Therefore, interpretation of electrochemical test results, such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), requires considerations of RE placement and bioreactor acclimation. In this workshop, I will show both from electrochemical and biological perspectives, how the RE and CE positions affect the CV and EIS characterization, using two different MFC configurations: spaced electrode configuration and separator electrode assembly configuration. I will also discuss the optimal RE position and methods to minimize errors in those electrochemical tests.

ACKNOWLEDGEMENTS

We would like to thank our sponsors, Office of Naval Research, Penn State Institutes of Energy and the Environment (PSIEE), Army Research Office, and International Society for Microbial Electrochemistry and Technology (ISMET), for their support.

We also appreciate the participation of all NA-ISMET attendees.

WORKSHOP ON "ELECTROCHEMICAL AND SPECTROSCOPIC ANALYSIS OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES (METs)"

On May 13, 2014, from 1:30-3:30 pm, we will hold a workshop prior to the start of the NA-ISMET conference related to different electrochemical and spectroscopic techniques used by researchers to study METs. There will be several presentations that delve into the analysis of these systems using electrochemical techniques such as electrochemical impedance spectroscopy, linear sweep voltammetry, and cyclic voltammetry, as well as analysis of biofilms using spectroscopic methods such as Raman spectroscopy. The presentations are conducted in a more informal, teaching-oriented style, with questions encouraged during the presentations. The presenters are listed below. There is no charge to attend the workshop, although seating may be limited. If you are interested in attending the workshop, please fill out the google form, at:

https://docs.google.com/forms/d/1gBC-hsJRCfMaGHB3LSufuTt2df-F8ZPzdhL6x9 NdFs/viewform.

The workshop location will be on the Penn State campus, at 135 Reber Building. If you need to contact someone regarding your reservation, please email Fang at fuz105@psu.edu

Presentations

Prof. Roland Cusick, University of Illinois at Urbana-Champaign, "Quantifying specific resistances at multiple interphases in microbial reverse electrodialysis cells using multichannel electrochemical impedance"

Dr. Jaroslaw (Yarik) Syzdek, Bio-Logic USA, "Electrochemical measurements – utilizing your hardware to the fullest"

Dr. Leonard Tender, Naval Research Laboratory, "Measurement of electron transport rates through electrochemically active biofilms"

Dr. Fang Zhang, Penn State University, "Reference and counter electrode positions affect electrochemical characterization of bioanodes in different bioelectrochemical systems"

Tours of the Penn State Environmental Engineering and Bioenergy Laboratories, May 13 and May 16, 2014

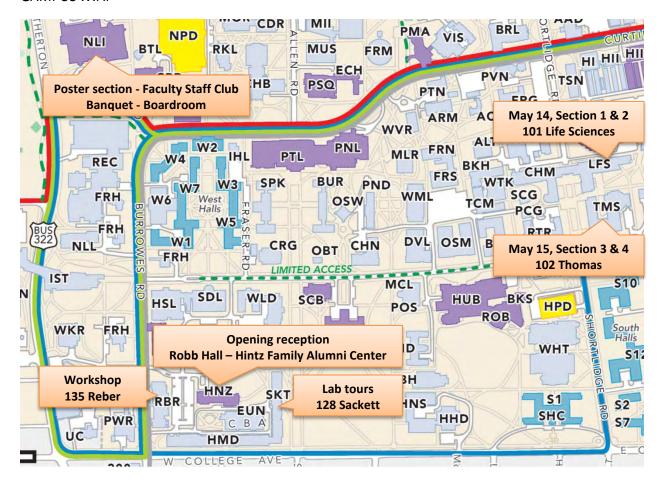
Attendees of the NA-ISMET meeting at Penn State University are invited to participate in a laboratory tour of the Environmental Engineering and Bioenergy laboratories. The focus of the tour will be the different microbial and electrochemical technologies being researched at Penn State (e.g. microbial fuel cells, microbial electrolysis cells and reverse electrodialysis systems). The time slots are 30 minutes long, with approximately 15 minutes for the tour with another 15 minutes allocated for additional discussion. Due to the size of the laboratories, the number of visitors will be restricted. Please sign up at our registration form to indicate what times you would be available to go on the tour, at

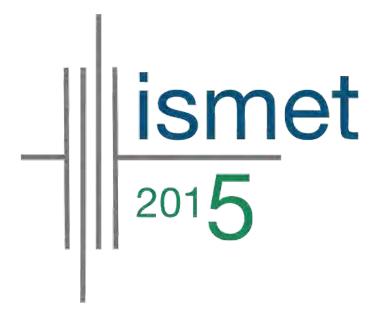
https://docs.google.com/forms/d/1 RqilhZstHCvnVkszyBQFrVOcPy4NeWgxJFEB234r88/viewform

You will obtain an email with assigned time slot later. If you need to contact someone regarding your lab tour registration, please email Hiroyuki Kashima at: hvk5121@psu.edu

Tours meet at Room 128 on the 1st floor of Sackett Building.

CAMPUS MAP







October 1-4, 2015

Arizona State University Tempe, Arizona, USA

PROGRAM BOOKLET



Welcome

On behalf of the organizing committee, I welcome you to ismet2015, the 5th international meeting on microbial electrochemistry and technologies. We organized this meeting based on the principle of the



International Society for Microbial Electrochemistry and Technologies – providing an opportunity for researchers from diverse backgrounds to meet and discuss the science and engineering of microbe-electrode interactions. With this in mind, take advantage of the multidisciplinary program we have at ismet2015, learn from novel areas outside your area of expertise and meet new people! Our ultimate goal is for you to generate new ideas and create new collaborations that can move our field forward. Enjoy your time at ASU!

- César Torres (Chair, Organizing Committee)

Internet access

To access wireless internet, connect to the network "asu guest", and follow the prompts to register.





Abstract access

All abstracts are available to view on the ismet2015 website. Please visit http://www.ismet2015.org from your laptop, tablet or phone, and enter the password provided in the program booklet during the meeting. Abstracts will be available on the ismet2015 website until December 31, 2015, and will then be available on the ISMET website for members.

Meals

As a part of the meeting, lunches are included on Friday, October 2, 2015, and Saturday, October 3, 2015, along with dinner on Saturday, October 3, 2015. Allergen information is available on the website at http://www.ismet2015.org.

Tours - Sunday, October 4, 2015

If you have registered to join tours to either the Desert Botanical Garden or Taliesin West, please gather outside Old Main after the end of the plenary session on Sunday. Members of the local organizing committee will help you board the bus to the venues. We may have a limited number of extra seats available if anyone who is not registered already for the tour wants to join. Please be prepared to pay by cash on spot to join.

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Thursday, October 1, 2015 - Workshop Session - Biodesign Institute

| | AL1-10/14 | B105 Auditorium |
|-------|--|---|
| | Workshop 1: | Workshop 2: |
| | Molecular biology and bioinformatics tools | Mathematical modeling |
| 12.00 | Check-in (Biodesig | n B Reception Area) |
| 12.30 | Jon Badalamenti , University of Minnesota, Twin Cities, USA | Andrew Marcus, Arizona State University, USA |
| 14.00 | Greg Caporaso , Northern Arizona University, USA | Cristian Picioreanu , Delft Institute of Technology, The Netherlands |

Thursday, October 1, 2015 - Plenary Session 1 - Old Main

| | Carson Ballroom |
|-------|---|
| 14.00 | Check-in |
| 16.00 | Welcome notes |
| | César Torres, Arizona State University, USA |
| | Korneel Rabaey, Ghent University, Belgium |
| 16.30 | Consideration of cathode specific surface area and hydrodynamics in scaling up microbial fuel cells |
| | Bruce Logan, Pennsylvania State University, USA |
| 17.00 | How do electrons get out of <i>Geobacter</i> ? It depends on how you ask |
| | Daniel Bond, University of Minnesota, Twin Cities, USA |
| 17.30 | Understanding native exoelectrogens and engineering new ones |
| | Caroline Ajo-Franklin, Lawrence Berkeley National Laboratory, USA |

Thursday, October 1, 2015 - Reception - Biodesign Institute

| | Lower Level Atrium |
|-------|-----------------------|
| 18.00 | Reception |
| 19.00 | ISMET awards ceremony |

Friday, October 2, 2015 – Morning Session – Memorial Union

| | Arizona Ballroom Session 1: Fundamentals of extracellular electron and | Turquoise Ballroom Session 2: Materials: Electrodes and membranes for |
|-------|--|---|
| | ionic transport | Materials: Electrodes and membranes for microbial electrochemical technologies |
| 8.50 | Invited talk: Biophysical investigations of extracellular electron transport in Shewanella oneidensis MR-1 Sahand Pirbadian, University of Southern California, USA | Invited talk: Investigating the behavior of single carbon granules as capacitive bioanodes in microbial fuel cells Annemiek ter Heijne, Wageningen University, The Netherlands |
| 9.20 | Temporal-spatial changes in viabilities and electrochemical properties of anode biofilms Bruce Logan, Pennsylvania State University, USA | Hydrophobic polyvinylidene fluoride (PVDF) membrane as a novel diffusion layer in activated carbon air cathode for microbial fuel cells Wulin Yang , <i>Pennsylvania State University</i> , USA |
| 9.40 | Heat of the moment: BioElectroCalorimetry reveals energetics of extracellular electron transfer Benjamin Korth, Helmholtz-Centre for Environmental Research, Germany | Application of gas diffusion and flow-through cathodes for carbon dioxide reduction in microbial electrosynthesis Suman Bajracharya, VITO, Belgium |
| 10.00 | Investigating the properties of Geobacter sulfurreducens DL-1 nanowires: Determining optimum condition of chemostat culturing for the best extracellular-electron-transfer performance Shiue-Lin Li, University of Southern California, USA | Designing cathodes for bioelectrochemical systems: Enzymatic vs. non-platinum catalysis for oxygen reduction Carlo Santoro, University of New Mexico, USA |
| 10.20 | Coffee | Break |
| 10.40 | Temperature dependency of long range electron transport in microbial biofilms Matthew Yates, Naval Research Laboratory, USA | High-performance nitrogen doped carbon aerogels air-cathodes for microbial fuel cells Xiaoyuan Zhang, Tshingua University, China |
| 11.00 | Electrochemical analysis of permselective properties of anodic biofilms in microbial fuel cells Naoufel Haddour, Université de Lyon, France | Reduced graphene oxide (rGO) fosters thick and highly-performing percolating electrochemically-active biofilms Bernardino Virdis, University of Queensland, Australia |
| 11.20 | Direct microbial electrosynthesis or hydrogen mediated microbial synthesis of acetate? Nicolas Bernet, INRA, France | Improved long term stability of air cathodes of microbial fuel cells through inhibition of cathodic microbial growth by enrofloxacin Weifeng Liu, Zhejiang University, China |
| 11.40 | The formation of electron conducting Geobacter sulfurreducens biofilms on graphite electrodes: Insights using proteomics Paul Kavanagh, National University of Ireland, Ireland | Performance of polyacrylonitrile (PAN)-based electrospun materials coated in PEDOT conductive polymer as electrodes for bioelectrochemical systems Juan Guzman, Cornell University, USA |
| 12.00 | Lunch (Venta | nna Ballroom) |

Friday, October 2, 2015 – Afternoon Session – Memorial Union

| | Arizona Ballroom | Turquoise Ballroom |
|-------|--|--|
| | Session 3: Novel microorganisms and synthetic biology approaches in microbial electrochemistry | Session 4: Design, modeling and optimization for scale-up of microbial electrochemical technologies |
| 13.20 | Invited talk: Mechanism of direct electron uptake in Methanococcus maripaludis Alfred Spormann, Stanford University, USA | Invited talk: Microbial wonderfuel cells: From nano-meter to cubic-meter Abraham Esteve-Núñez, University of Alcalá, Spain |
| 13.50 | Electrically conductive nanofilaments induction in Rhodopseudomonas sp. Krishnaveni Venkidusamy, University of South Australia, Australia | 90-liter pluggable microbial fuel cell for brewery wastewater treatment and energy harvesting Yue Dong , Harbin Institute of Technology, China |
| 14.10 | Regulation of microbe-electrode hybrid system with genetically engineered microorganism for developing novel fermentation process Jung Rae Kim, Pusan National University, South Korea | Strategies for high coulombic efficiency in microbial electrochemical systems for efficient energy recovery from wastewater Tom Sleutels, Wetsus, The Netherlands |
| 14.30 | Capturing complete genomes of novel halophilic metal reducers from the deep subsurface: Short and long read metagenomics of Soudan Mine communities enriched on electrodes Jon Badalamenti, University of Minnesota, Twin Cities, USA | Combination of a bioanode and a biocathode in a bioelectrochemical system allows a more than two-fold increase in energetic efficiency of microbial electrosynthesis Elie Desmond-Le Quéméner, IRSTEA-HBAN, France |
| 14.50 | | Break |
| 15.10 | Characterization and improvement of the catalytic activity of <i>C. ljungdahlii</i> in a microbial electroreduction application Thomas Kirchner, RWTH Aachen University, Germany | Microbial desalination cell operation: Start-up protocol and batch mode analysis Juan Ortiz, FCC Aqualia, Spain |
| 15.30 | Extracellular electron transfer pathways in Gram-positive bacteria Feng Zhao, Chinese Academy of Sciences, China | Using bio/abio hybrid electrodes in a two- chamber microbial electrolysis cell for protons in situ use and hydrogen production under periodic reversion of polarity Yong Jiang, Tshingua University, China |
| 15.50 | Extending the applicability range for bioelectrochemical systems: Prospects for alkaline exoelectrogenesis Juan Antonio Baeza, Universitat Autònoma de Barcelona, Spain | Bringing CO ₂ -based microbial electrosynthesis closer to practical implementation Ludovic Jourdin , <i>University of Queensland</i> , Australia |
| 16.10 | The effect of pH and buffer concentration on anode respiration in <i>Thermincola ferriacetica</i> Bradley Lusk , <i>Arizona State University</i> , USA | A microbial fuel cell steady state electrical model based on <i>Geobacter sulfurreducens</i> strains Carlos Hernández, Universidad Nacional de Colombia, Colombia |
| 16.40 | Poster Session 1 (| (Arizona Ballroom) |

Saturday, October 3, 2015 – Morning Session – Memorial Union

| | Arizona Ballroom | Turquoise Ballroom |
|-------|---|--|
| | Session 5: | Session 6: |
| | New applications of microbial electrochemical technologies | Studies on microbial and enzymatic pathways in microbial electrochemistry |
| 8.50 | Invited talk: Microbial electro-concentration for nutrient recovery from urine Stefano Freguia, University of Queensland, Australia | Invited talk: Anaerobic oxidation of methane coupled with extracellular electron transfer to conductive solids Hyung-Sool Lee, University of Waterloo, Canada |
| 9.20 | Electrochemically-driven fermentation of organic substrates with undefined mixed microbial cultures Marianna Villano, Sapienza University of Rome, Italy | Using RNA-seq to study electron transfer pathways and carbon fixation in a marine biocathode community Brian Eddie, Naval Research Laboratory, USA |
| 9.40 | Hydrogen as an electron donor for copper removal in bioelectrochemical systems Pau Rodenas , Wageningen University, The Netherlands | Flavin-mediated electron transfer increases current production by exoelectrogenic Escherichia coli to rival Shewanella oneidensis MR-1 Michaela TerAvest, Michigan State University, USA |
| 10.00 | Integrated production, extraction and concentration of acetic acid from CO ₂ through microbial electrosynthesis Sylvia Gildemyn, Ghent University, Belgium | Sorting the logistics of electron transfer within and across the periplasmic space of Shewanella oneidensis MR-1 Catarina Paquete, Universidade Nova de Lisboa, Portugal |
| | | |
| 10.20 | Coffee | e Break |
| 10.20 | Coffee | Session 8: |
| 10.20 | Coffee | |
| 10.40 | Tainted love: Integrating electroorganic reactions with microbial transformations Falk Harnisch, Helmholtz-Centre for Environmental Research, Germany | Session 8: Wastewater treatment and other |
| | Tainted love: Integrating electroorganic reactions with microbial transformations Falk Harnisch, Helmholtz-Centre for | Session 8: Wastewater treatment and other bioremediation and biosensing applications Combined COD and nitrogen removal in bioelectrochemical systems Boris Tartakovsky, National Research Council of |
| 10.40 | Tainted love: Integrating electroorganic reactions with microbial transformations Falk Harnisch, Helmholtz-Centre for Environmental Research, Germany Hydrodynamic chronoamperometry for probing kinetics of anaerobic microbial metabolism | Session 8: Wastewater treatment and other bioremediation and biosensing applications Combined COD and nitrogen removal in bioelectrochemical systems Boris Tartakovsky, National Research Council of Canada, Canada When bioelectrochemical systems meet forward osmosis: Accomplishing wastewater treatment and reuse through synergy Zhen He, Virginia Polytechnic Institute and State |
| 10.40 | Tainted love: Integrating electroorganic reactions with microbial transformations Falk Harnisch, Helmholtz-Centre for Environmental Research, Germany Hydrodynamic chronoamperometry for probing kinetics of anaerobic microbial metabolism Antonin Prévoteau, Ghent University, Belgium Electropotential driven semi-artificial photosynthesis in biocatalyzed photoelectrochemical system G. N. Nikhil, CSIR-Indian Institute of Chemical Technology, India Oil production from complex substrates with microbiomes at ambient temperatures and pressures: Electrochemically induced phase separation in the carboxylate platform Jiajie Xu, Cornell University, USA | Session 8: Wastewater treatment and other bioremediation and biosensing applications Combined COD and nitrogen removal in bioelectrochemical systems Boris Tartakovsky, National Research Council of Canada, Canada When bioelectrochemical systems meet forward osmosis: Accomplishing wastewater treatment and reuse through synergy Zhen He, Virginia Polytechnic Institute and State University, USA Integrated bioelectrochemcial systems in biorefinery for sustainable bioenergy production Abhijeet Borole, Oak Ridge National Laboratory, |

Saturday, October 3, 2015 – Afternoon Session – Memorial Union

| | Arizona Ballroom Session 7: Microbial ecology of mixed culture | Turquoise Ballroom Session 8: Wastewater treatment and other |
|-------|--|--|
| 13.20 | microbial electrochemistry systems Invited talk: Characterizing an autotrophic biocathode microbial consortia using electrochemistry, spectroscopy, and metaomics Sarah Strycharz-Glaven, Naval Research Laboratory, USA | Invited talk: Integration of advances in material science with microbial electrolysis cells for resource recovery from dilute organic streams Pascal Saikaly, King Abdullah University of Science & Technology, Saudi Arabia |
| 13.50 | Exploring the brine pool in the red sea as a source of exoelectrogenic communities Noura Shehab, King Abdullah University of Science and Technology, Saudi Arabia | Nitrogen removal and electricity generation from domestic wastewater using flat-panel aircathode microbial fuel cells Taeho Lee , Pusal National University, South Korea |
| 14.10 | Functional and taxonomic dynamics during long-term bioelectrochemical systems operation Orianna Bretschger, J. Craig Venter Institute, USA | The effect of inoculum and cathode potential on bioelectrochemical autotrophic sulfate reduction process Guillermo Pozo, University of Queensland, Australia |
| 14.30 | Change in bacterial community structure from natural extreme environments following bioelectrochemical system incubation Ignacio Vargas, Pontificia Universidad Católica de Chile, Chile | Filtering with an MFC anode: A new concept for the integration of microbial fuel cells into membrane bioreactors Joana Danzer, University of Freiburg, Germany |
| 14.50 | Coffee | Break |
| 15.10 | Electromicrobiology insights with functionalized electrode surfaces Krishna Katuri, King Abdullah University of Science and Technology, Saudi Arabia | Integration of electrochemical techniques for a complete treatment of industrial wastewater: electrocoagulation plus microbial electrochemical fluidized bed reactors Sara Tejedor-Sanz, Universidad de Alcalá, Spain |
| 15.30 | Metagenomic analysis for electrogenic microbial communities adapted to different surface potentials Shun'ichi Ishii, Japan Agency for Marine-Earth Science and Technology, Japan | Sustainable hydrogen production from agro- industrial wastewater combining dark fermentation and microbial electrolysis Olga Ayala, Universidad de Guadalajara, Mexico |
| 15.50 | Can exoelectrogens outcompete non- exoelectrogens in microbial fuel cell? Ningshengjie Gao, Oregon State University, USA | Power generation and domestic wastewater treatment using microbial fuel cell and anaerobic fluidized membrane bioreactor (MFC-AFMBR) system under different hydraulic retention times Kyoung-Yeol Kim, Pennsylvania State University, USA |
| 16.20 | Poster Session 2 (| (Arizona Ballroom) |
| 18.30 | | ana Ballroom) |

Sunday, October 4, 2015 - Plenary Session 2 - Old Main

| | Carson Ballroom |
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| 9.30 | Bioelectrochemical systems integrated with water desalination and nutrient recovery |
| | Xia Huang, Tsinghua University, China |
| 10.00 | Synthetic electromicrobiology: Novel electronics, sensors, and bioenergy strategies from a basic |
| | understanding of extracellular electron exchange |
| | Derek Lovley, University of Massachusetts, USA |
| 10.30 | How useful is electricity as driver of microbial processes? |
| | Korneel Rabaey, Ghent University, Belgium |
| 11.00 | Concluding remarks, ismet2015 awards ceremony and group photograph |
| | Bruce Rittmann, Arizona State University, USA |

List of Posters

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| 1-2 | Engineering electrochemically active bacterium <i>Shewanella oneidensis</i> MR-1 for production of 3-hydroxypropionic acid from glycerol Ganapathiraman Munussami , <i>Pusan National University</i> , <i>South Korea</i> |
| | |
| 1-3 | Electrons à la carte: fine-tuning of exoelectrogenic bacteria using synthetic biology Beatrice Vetter , <i>University of Edinburgh</i> , <i>UK</i> |
| 1-4 | Characterization of the electrical current generation capabilities of the thermophile Thermoanaerobacter pseudethanolicus, using xylose, cellobiose, glucose, or acetate in a poised anode Bradley Lusk, Arizona State University, USA |
| 1-5 | In situ genetic modification of a biocathode community |
| . 0 | Nicholas Kotloski, Naval Research Laboratory, USA |
| 1-6 | Characterization of electron transfer kinetics from donor substrate to the anode: Implication of |
| | biofilm conductivity |
| | Bipro Dhar, University of Waterloo, Canada |
| 1-7 | The effect of adhesive pili on the interaction between graphite electrodes and electrically active Escherichia coli |
| | Michael Lienemann, VTT Technical Research Center of Finland Ltd., Finland |
| 1-8 | A molecular view of the outer membrane/flavin interactions and the relationship to bacterial extracellular electron transfer in Shewanella spp. Sofia Babanova, University of New Mexico, USA |
| 1-9 | Transcriptional regulation of outer membrane cytochrome genes <i>omcA</i> and <i>mtrC</i> by two CRP-dependent promoters in <i>Shewanella oneidensis</i> MR-1 Takuya Kasai , <i>Tokyo University of Pharmacy and Life Sciences, Japan</i> |
| 1-10 | Electrical niches of Geobacteraceae |
| | Lori Zacharoff, University of Minnesota, Twin Cities, USA |
| 1-11 | The effect of pH on electrochemical response of anode biofilms of Geobacter sulfurreducens |
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| 1-12 | Metabolic flux analysis with bioelectricity generation from glucose by <i>Klebsiella pneumoniae</i> L17 in microbial fuel cell |
| | Changman Kim, Pusan National University, South Korea |

| 1-13 | Directing inward electron transport to the cytosol in Mtr-Escherichia coli using fumarate reductase Moshe Baruch , Lawrence Berkeley National Laboratory, USA |
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| 1-14 | Multiple electron transport pathways for extracellular respiration to electrodes in biofilms of Geoalkalibacter ferrihydriticus |
| | Rachel Yoho, Arizona State University, USA |
| 1-15 | Bioelectrogenic reduction towards hydrogen production in microbial electrolytic cell: Correlation of redox mediators generated and hydrogenase gene expression G. N. Nikhil, CSIR-Indian Institute of Chemical Technology, India |
| 1-16 | Terminal electron acceptor (oxygen) availability influence on microbial fuel cell performance operating in air-cathode mode G. N. Nikhil, CSIR-Indian Institute of Chemical Technology, India |
| 1-17 | Surface enhanced confocal raman microscopy time series of a AgNp bio-precipitating Shewanella biofilm Falk Harnisch, Helmholtz Center for Environmental Research – UFZ, Germany |
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| 1-18 | Effects of NaCl concentration on anode microbes in microbial fuel cells Morio Miyahara, Tokyo University of Pharmacy and Life Sciences, Japan |
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